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THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG[1]

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I. THE COMPLEXITY OF ORGANIZATION OF THE INSECT EGG

1. *Introduction*

THE morphological and experimental investigations of the germ-cell cycle in insects which the writer has carried on during the past ten years have resulted in the accumulation of many data which indicate the complexity of organization of the eggs of these animals, and suggest hypotheses regarding the nature and genesis of this organization. That the animal egg at the time development begins does not consist of a homogeneous mass of protoplasm, as the old theory of epigenesis required, but is a highly organized cell containing various kinds of protoplasm localized in definite regions has been proved conclusively by numerous investigators working with the eggs of many different species. The degree of organization at the time of fertilization varies according to the species of animal, but all embryologists admit that the insect egg is one of the most highly organized of all.

The nature and genesis of the different kinds of protoplasm in the insect egg is the principal problem discussed in the following pages. This problem is the logical successor of those dealing with cell lineage and the organization of the egg at the time development begins. The re-

[1] Presented to the Johns Hopkins Scientific Association on October 9, 1917.

lations between this problem and the larger problem of heredity and development are very close indeed. The study of heredity is concerned not only with the adult animal, but also with every stage in the development of the adult since the fertilized egg, or embryo, or larva that arises from it, is an individual just as is the fully developed animal. The organization of the fertilized egg is the result of the processes of differentiation that take place at each stage in the history of the egg and sperm from the time the primordial germ cells are segregated until the highly specialized gametes have become fully formed. The period extending from the formation of the primordial germ cells to and through the growth period of the gametes is one of the least known in the entire history of the individual. It is nevertheless a most important period, for during this time, at least in the insect egg, the principal axes of the individual are established and different kinds of cytoplasm are elaborated and localized that are predetermined to form definite parts of the embryo.

The stages in the germ-cell cycle in insects belonging to different orders, families, genera or species are often quite different, as is to be expected, hence the data on which this paper is based were derived from the study of a number of species. On this account it seems best to give an abridged description of the germ-cell cycle in one group and include, wherever necessary, data derived from the study of other groups. The principal work has been carried on with representatives of the orders Diptera, Hymenoptera and Coleoptera, and of these, the order Coleoptera has furnished the best material for experimental purposes. We will select, therefore, the eggs of certain chrysomelid beetles for descriptive purposes.

2. *The Structure of the Insect Egg at the Time of Deposition*[1]

At the time of deposition (Fig. 1) the beetle's egg consists largely of deutoplasm—a substance which is used

[1] Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

up during the growth of the embryo. This deutoplasm is composed of vitelline spheres, which contain refringent granules, the vitelline bodies, and of oil globules. The deutoplasmic bodies are embedded in a viscid cytoplasmic matrix which, however, is very slight in amount as compared with the deutoplasmic material. At the periphery of the egg is a thin cortical layer of cytoplasm which is continuous with the cytoplasm in which the vitelline spheres lie. A short distance back of the anterior pole of the egg is a thickening of this cortical layer in which the maturation divisions of the oocyte nucleus occur. The cytoplasm appears to be homogeneous except at the posterior end, at which place, in many insects, inclusions have been discovered which appear to play a rôle in the formation of the primordial germ cells.[2]

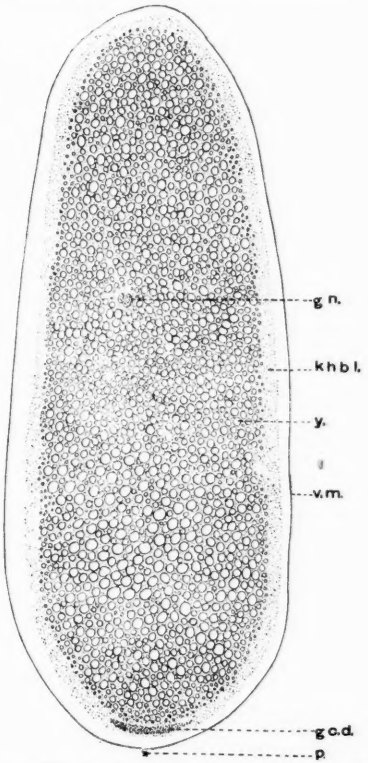

FIG. 1. Longitudinal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd* = germ-cell determinants. *gn* = germ-nuclei copulating. *khbl* = cortical layer of cytoplasm. *p* = posterior end. *vm* = vitelline membrane. *y* = yolk. (From Hegner, 1909.)

These inclusions in the chrysomelid beetles take the form of a polar disc of granules which I have called germ-cell, keimbahn, or germ-line determinants.[3] Similar inclusions (Fig. 2) have been

[2] Hegner, 1909, *Journ. Morph.*, Vol. 20.

[3] Hegner, 1908, *Biol. Bull.*, Vol. 16.

[4] Weisman, 1863, *Zeit. f. wiss. Zool.*, Bd. 13; Metschnikoff, 1866, *ibid.*,

noted in Diptera,[4] in parasitic Hymenoptera,[5] and in Hymenopterous gall flies.[6] There should also be mentioned in this connection minute bodies that have been

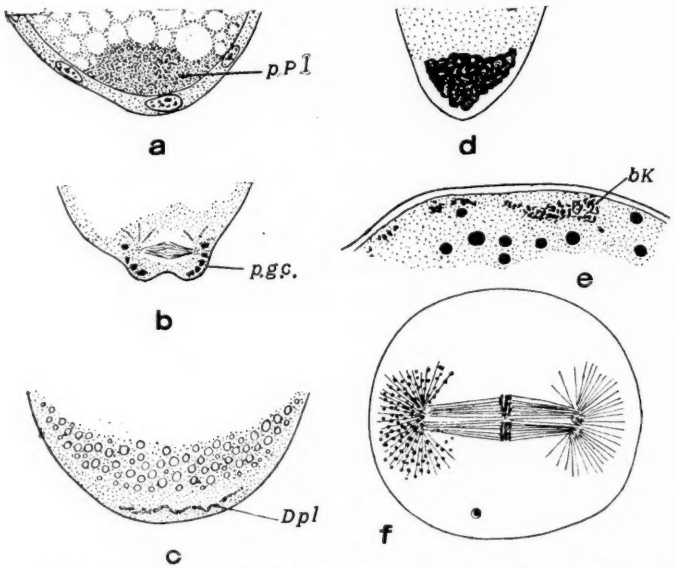

FIG. 2. Germ-line determinants in the eggs of various animals. *a*. Polеplasm (*p Pl*) at the posterior end of the egg of *Miastor* (Hegner, 1914). *b*. Keimbahnplasma (*pgc*) at the posterior end of the egg of *Chironomus*. One of the four cleavage nuclei in undergoing mitosis. (Hasper, 1911.) *c*. Dotterplatte (*Dpl*) at the posterior end of the egg of *Calliphora*. (Noack, 1901.) *d*. Germ-line determinant at the posterior end of the egg of *Apanteles*. (Hegner, 1915.) *e*. Besondere Körper (*bK*) in the egg of *Sagitta*. (Elpatiewsky, 1909.) *f*. Ectosomen at one end of the first cleavage spindle in the egg of *Cyclops*. (Amma, 1911.)

discovered in the cytoplasm of the eggs of the carpenter ant, *Camponotus*, and in those of various other insects.[7]

Bd. 16; Ritter, 1890, *ibid.*, Bd. 50; Noack, 1901, *ibid.*, Bd. 70; Kahle, 1908, *Zoologica*, Bd. 21; Hasper, 1911, *Zool. Jahrb.*, Bd. 31; Hegner, 1912, *Science*, Vol. 36; Hegner, 1914, *Journ. Morph.*, Vol. 25.

[5] Silvestri, 1906, *Boll. Labor. Zool. R. Sc. Agr. Portici*, Vol. 1; Silvestri, 1908, *ibid.*, Vol. 3; Silvestri, 1914, *Anat. Anz.*, Bd. 47; Silvestri, 1915, *Boll. Labor. Zool. R. Sc. Agr. Portici*, Vol. 10; Silvestri, 1916, *Rend. D. R. Accad. D. Lincei*, Vol. 25; Martin, 1914, *Zeit. f. wiss. Zool.*, Bd. 110; Hegner, 1914, *Anat. Anz.*, Bd. 46; Hegner, 1915, *Journ. Morph.*, Vol. 26.

[6] Hegner, 1915, *Journ. Morph.*, Vol. 26.

[7] Blochmann, 1886, *Festsch. nat.-med. Verein zu Heidelberg;* Sule, 1906,

These have been considered symbiotic bacteria, but their true nature remains yet to be definitely established.

3. *Cleavage*

The first cleavage nucleus of the beetle's egg lies somewhat anterior of the center in a small island of cytoplasm that is continuous with the cytoplasm that surrounds the deutoplasmic bodies (Fig. 1, *gn*). During early cleavage no cell walls are formed, but after each division the daughter nuclei move a short distance apart and then divide again. Successive divisions and migrations of the cleavage nuclei (Fig. 3) finally result in the production of

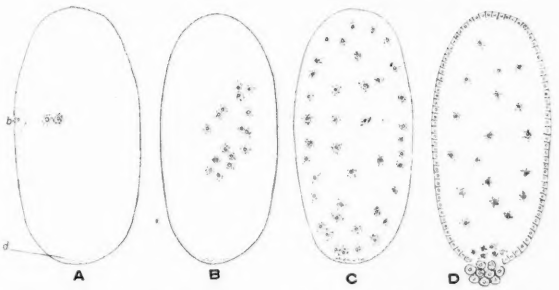

FIG. 3. Diagrams showing four stages in the cleavage and blastoderm formation of the chrysomelid beetle's egg. *a*. Two-cell stage. *b* = polar bodies. *d* = pole disc. *b*. Sixteen-cell stage. *c*. Just before formation of blastoderm. *d*. Blastoderm stage. At the posterior end are the primordial germ cells. (Hegner, 1909, 1914.)

hundreds of nuclei, which come to lie just beneath the cortical layer of cytoplasm, and are each surrounded by an irregular mass of cytoplasm. The fusion of these cytoplasmic masses with the cortical layer then takes place, followed by the intervention of cell walls, thus forming a blastoderm of a single layer of cells, each of which contains a cleavage nucleus, part of the cytoplasm which it brought to the periphery with it, and a portion of the

Stzber. böhm. Gesellsch. Wiss. Prag.; Sulc, 1910, *ibid.;* Mercier, 1907, *Arch. Protistenk.*, Bd. 9; Pierantoni, 1910, *Zool. Anz.*, Bd. 36; Buchner, 1912, *Arch. Protistenk.*, Bd. 26; Tanguary, 1913, *Bull. Ill. St. Lab. Nat. Hist.*, Vol. 9; Hegner, 1915, *Journ. Morph.*, Vol. 26.

cortical layer. Not all of the cleavage nuclei take part in blastoderm formation, many of them remaining behind in the yolk to aid in breaking down this substance.

4. *The Origin of the Primordial Germ Cells*

Blastoderm formation is interrupted at the posterior end of certain chrysomelid beetles' eggs by the segregation of the primordial germ cells (Fig. 4). Those cleav-

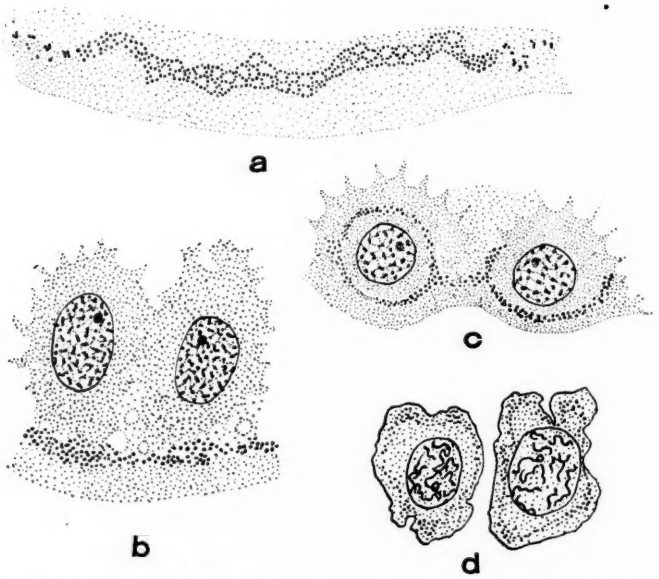

FIG. 4. Portions of the posterior end of eggs of *Calligrapha* showing stages in the formation of the primordial germ cells. *a*. Pole-disc granules in the cortical layer. *b*. Two cleavage nuclei just before the pole disc is reached. *c*. Two cleavage nuclei becoming surrounded by pole disc granules. *d*. Two primordial germ cells entirely separated from the egg. (Hegner, 1909.)

age nuclei that encounter the granules of the pole-disc do not produce blastoderm cells, but continue their migration and are finally cut off from the rest of the egg as distinct cells. These are the primordial germ cells, of which there are sixteen. Each of these cells contains a portion of the cortical layer that includes pole-disc gran-

ules, thus differing in content from the blastoderm cells. They differ also from blastoderm cells in size, being considerably larger. This is probably due at first to the inclusion of the pole-disc granules, but later a greater difference in size is brought about by the failure of the germ cells to divide as rapidly as do the blastoderm cells.

An early origin of the primordial germ cells in a similar manner has been described in a number of insects besides Coleoptera, especially in the midge, *Chironomus,*[8] where these cells are derived from one of the cleavage nuclei at the four-cell stage; in the pedogenetic larva of the fly, *Miastor,*[9] where one of the first eight cleavage nuclei becomes the nucleus of the primordial germ cell, in the fly, *Calliphora,*[10] and in parasitic Hymenoptera.[11]

5. *The Formation of the Ovaries*

In chrysomelid beetles, *Chironomus, Miastor* and certain other species of insects, the primordial germ cells undergo a multiplication period shortly after they are formed. This is followed by a period during which they become lodged within the embryo—either by the shifting of the surrounding tissues or by migration or by both these processes. At this time also they become separated into two groups; in chrysomelid beetles each group appears to contain thirty-two germ cells; in *Miastor* each consists of four germ cells. One group becomes located on either side of the embryo and later gives rise to one half of the germ glands. The sex of the individual can be determined by the morphology of the germ glands before the young hatches.

The further history of the germ cells in female insects is in general as follows. From each primitive ovary a number of ovarian tubules arise each containing many germ cells (oogonia) which have undergone a multiplication period. The oogonia finally cease dividing and the

[8] Hasper, 1911, *Zool. Jahrb.,* Bd. 31.
[9] Kahle, 1908, *Zoologica,* Bd. 21; Hegner, 1914, *Journ. Morph.,* Vol. 25.
[10] Noack, 1901, *Zeit. f. wiss. Zool.,* Bd. 70.
[11] Silvestri, *l. c.*

ultimate oogonia are ready to enter upon the growth period. A period of differentiation may or may not intervene, according to the species, during which nurse cells are formed. When the oocytes have reached their full size they separate from the ovarian tubule, pass down the oviduct into the vagina and are deposited. Each egg is surrounded by two membranes; a thin inner vitelline membrane and a thicker, outer membrane, the chorion.

6. *The Complexity of Organization of the Insect Egg.*

(*a*) *Comparison between Eggs of Insects and Those of Other Animals.*—Insect eggs differ greatly from those usually employed for the study of egg organization, since they are, as a rule, laid in the air and not in the water, and because cleavage is of the superficial type, cell walls being absent until a comparatively late cleavage stage. The eggs of chrysomelid beetles are particularly favorable for study, since they may be subjected to the most violent experimental conditions without preventing their development.[12]

In insect eggs the character of the blastoderm cells depends, as in holoblastic eggs, upon the kinds of protoplasm they contain, but all those phenomena connected with the position of the cleavage spindle, which have been so carefully studied in the eggs of mollusks, worms, ascidians and other animals, can have no influence upon the localization of different substances in various parts of an insect egg, because in the latter the volume of the egg is thousands of times greater than that of the cleavage spindle. Furthermore in holoblastic eggs differentiated substances are segregated in different cells during early cleavage and are there isolated by cell walls, and to this isolation is attributed in large part the progress of differentiation; but in the insect egg the different kinds of cytoplasm are in direct continuity until hundreds of cleavage nuclei are present, and are not separated by cell walls until the blastoderm is fully formed.

[12] Hegner, 1908, *Biol. Bull.*, Vol. 16; 1909, *Journ. Exp. Zool.*, Vol. 6; 1911, *Biol. Bull.*, Vol. 20.

(*b*) *Results of Experiments with Gravity and Centrifugal Force.*—A chrysomelid beetle, such as *Calligrapha* or *Leptinotarsa,* during the process of egg-laying clings to the under side of a leaf and the end of the egg that emerges first is glued to the leaf by a viscid secretion. Then the egg is pushed back away from the abdomen and another is laid[13] (Fig. 5). In this way from four to

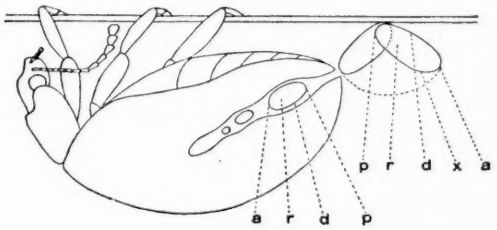

FIG. 5. Diagram showing a chrysomelid beetle, *Calligrapha bigsbyana,* clinging to the underside of a willow leaf and laying her eggs. The relation between the orientation of the egg before and after deposition is indicated by the letters. *a* = anterior. *d* = dorsal. *l* = left. *p* = posterior. *r* = right. *x* = anterior ventral surface where a spot of India ink was placed as a guide for orienting the eggs during experiments. (Hegner, 1909.)

eighty eggs are laid in one group within a period of about an hour. These eggs hatch in approximately five days. A few hours before they hatch the young can be seen distinctly through the semi-transparent egg shell. An examination of hundreds of eggs at this stage in their development has established the fact that the posterior end of the egg is attached to the leaf and the anterior end is free. In every other respect the orientation of the young in the egg corresponds to that of the egg as it lay within the body of the mother before deposition; that is, the ends and various surfaces of the egg are definitely determined before deposition and correspond to the orientation of the mother as indicated in the diagram (Fig. 5). This rigid correspondence between the orientation of the egg and that of the adult is known as the "law of orientation" which was first discovered by Hallez in 1886.

Since the eggs of these beetles are usually attached to

[13] Hegner, 1909, *Journ. Exp. Zool.,* Vol. 6.

the underside of leaves, it was suggested that the orientation of the young might depend upon the force of gravity, but eggs that were first marked with India ink and then placed in every conceivable position with respect to gravity proceeded to develop as though undisturbed.[14] It seemed from this, therefore, that the position of the young must be predetermined in the undeveloped egg.

Several kinds of experiments were performed in order to discover the complexity and fixity of this apparent organization. First, the eggs were subjected to a force greater than gravity by means of a centrifugal machine. Hundreds of eggs were revolved at different rates of speed for various lengths of time and in many different positions. A description of one experiment will serve to illustrate the results obtained.[15] In this experiment freshly laid eggs were placed in cavities in a block of paraffin with the posterior end toward the center of rotation, and were revolved in a hydraulic centrifuge for sixteen hours. The heavier substances were thrown to the outer end and the lighter protoplasm accumulated at the inner end, where an embryo developed (Fig. 6). It is perfectly evident that the protoplasm from the various parts of the egg has, in its new position, developed into the tissue that it would have given rise to if it had been left undisturbed. Normally the yolk would be surrounded by the embryonic tissue and would be en-

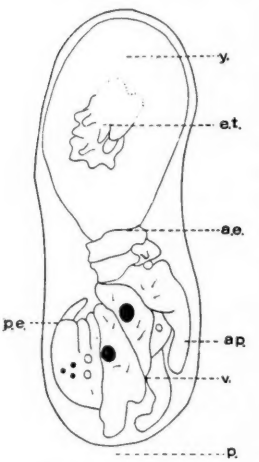

FIG. 6. Side view of a freshly laid egg of *Calligrapha multipunctata*, which was centrifuged for sixteen hours and then taken out and allowed to develop for nine days. *ae* = anterior end of embryo. *ap* = appendage of thorax. *et* = embryonic tissue. *p* = posterior end of egg. *pe* = posterior end of embryo. *v* = ventral. *y* = yolk. (Hegner, 1909.)

[14] Wheeler, 1889, *Journ. Morph.*, Vol. 3; Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

[15] Hegner, 1909, *Journ. Exp. Zool.*, Vol. 6.

closed by the mid-intestine, but in this case a dwarf embryo has developed without growing around the nutritive material.

The effects of centrifugal force upon insect eggs are different from those produced upon the other types of eggs that have been employed for such experiments. In the eggs of worms,[16] mollusks,[17] etc., apparently the materials that undergo stratification under the influence of centrifugal force have no influence upon the "ground substance" which is "the seat of polarity and pattern of organization of the cell." In the insect egg, the organized protoplasm is almost entirely limited to the cortical layer and this layer may be shifted away from the periphery by a sufficient force and may become massed at the inner light end when an undeveloped egg is centrifuged.

Since the cytoplasm develops in its new situation and proceeds to build up an embryo as nearly normal as is possible under the conditions imposed upon it, it is evident that the potencies of the cytoplasmic areas are predetermined at the time the egg is laid.

It was hoped by means of these experiments with centrifugal force to throw the pole-disc granules and the cytoplasm containing them into some other part of the egg. If the germ cells arose from this material in its new position the conclusion would have been convincing that these substances were necessary for the formation of the reproductive cells. Unfortunately, although the cortical layer at the posterior end was shifted by the centrifugal force, it was impossible to locate accurately the germ cells in the embryos that developed from the eggs that were thus operated upon.

(*c*) *Relation between Cleavage Nuclei and Egg Organization.*—During the course of my early studies of chrysomelid eggs it occurred to me that the nuclei that result from early cleavage might be definite in number and in

[16] Lillie, F. R., 1906, *Journ. Exp. Zool.*, Vol. 3.
[17] Conklin, 1917, *Journ. Exp. Zool.*, Vol. 22.

distribution and that they might be qualitatively different. If this were true, then nuclei of one sort might always migrate into one part of the egg and might determine the nature of the tissue that developed there, and nuclei of other sorts might likewise become located in other predetermined parts of the egg. Careful studies of the origin and migration of the cleavage nuclei[18] have led to the conclusion that the distribution of these nuclei is adventitious and that they are all potentially alike—that is are totipotent—a view that is now held by most embryologists regarding the relative importance of nucleus and cytoplasm during cleavage. That the nuclei may play a part in the differentiation of the cortical layer of cytoplasm during the cleavage period is highly improbable, since definite cytoplasmic organization already exists before cleavage begins. The factors brought into the egg by the spermatozoon, however, have an opportunity at this time to modify the initial organization and thus the early embryo may exhibit paternal characteristics. Whether or not such an influence is exerted at this time is not known.

The kind of tissue that develops from any part of the egg, therefore, depends upon the kind of cytoplasm encountered by the nuclei.

(*d*) *Complexity of Organization as Indicated by the Development of Parts of Eggs.*—More convincing evidence of the presence of a complex and fixed organization in the cytoplasm was derived from operations performed upon eggs with a hot needle. Parts of the freshly deposited eggs were killed by being touched with a hot needle and these parts were thus prevented from taking part in development. The living portions of the eggs continued to develop and in every case produced those parts of the embryo that they would have formed if the egg had not been injured.[19] This seems to prove that every part of the egg cytoplasm is set aside for the pro-

[18] Hegner, 1914, *Journ. Morph.*, Vol. 25.

[19] Hegner, 1911, *Biol. Bull.*, Vol. 20.

duction of a definite part of the embryo, and hence of the larva and adult, and that the cytoplasm is therefore highly organized at the time the egg is fertilized. After such experiments there is no regeneration of substances.

As stated above, the cortical layer of cytoplasm is visibly alike throughout except at the posterior end, where it has embedded in it the pole-disc granules. One of the most interesting results of the operations performed with the hot needle was obtained by killing the posterior portion of the egg containing the pole-disc (Fig. 7). Eggs

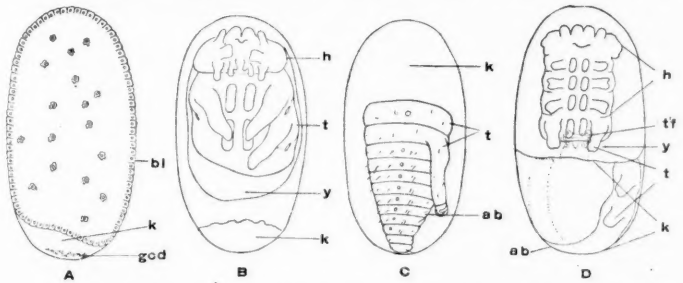

FIG. 7. Diagrams showing the results of killing parts of the eggs of *Leptinotarsa decemlineata* with a hot needle. (Hegner, 1911.)

a. Longitudinal section through an egg twenty-four hours old. The posterior end (*k*) was killed just after the egg was deposited (conditions as in Fig. 1). No germ cells were produced.

b. Ventral view of an embryo three days old. The posterior end (*k*) was killed just after the egg was deposited. The part that remained alive gave rise to the head and about half of the thorax.

c. Side view of an egg five days old. The anterior end (*k*) was killed in the blastoderm stage (Fig. 3, *d*). The part that remained alive produced the abdomen (*ab*) and about half of the thorax (*t*).

d. Ventral view of an egg three days old. The posterior end (*k*) was killed when the embryo was two days old. The anterior half continued to develop. The independence of the tissues is indicated by the minute end of the tail-fold (*tf*) which developed normally after being separated from the rest of the embryo. The two parts of the egg underwent a revolution of ninety degrees during the twenty-four hours succeeding the experiment.

ab = abdomen. *bl* = blastoderm. *gcd* = pole-disc. *h* = head. *k* = portion of egg killed with hot needle. *t* = thorax. *tf* = tail fold. *y* = yolk.

thus modified produced embryos without germ cells, proving that this cytoplasmic region is necessary for their formation. The castration of the individual may also be performed in a similar fashion after the germ cells have been extruded from the egg (Fig. 3, *d*), and it is interesting to note that sexless chicks have recently been pro-

duced in a similar fashion, by removing the region of the embryo from which the germ cells arise.[20]

The blastoderm is of course definitely organized, since its cells contain organized cytoplasm, and by killing parts of the eggs in the blastoderm and later stages results were obtained similar to those produced when fresh eggs were operated upon (Fig. 7, *b*, *c*, *d*).

7. *Summary of Part I*

Summarizing the data briefly given above, we may say:

1. Morphological and experimental studies have proved that the eggs of animals are more or less highly organized at the time of fertilization.

2. We know almost nothing about the nature and genesis of this organization.

3. Descriptions are given of the condition of the eggs of certain chrysomelid beetles at the time of deposition, of the stages of cleavage and blastoderm formation, of the origin of the germ cells, and of the principal stages in the germ-cell cycle.

4. The eggs of certain chrysomelid beetles and of other insects are definitely organized when deposited as indicated by observations on normally developing eggs and by experiments with gravity.

5. This organization exists in the cytoplasm as indicated by a morphological study of cleavage, by experiments with gravity and centrifugal force, and by killing with a hot needle parts of eggs in various stages of development.

6. These observations and experiments prove also that the nuclei up to the time of blastoderm formation are totipotent.

II. THE GENESIS OF THE ORGANIZATION OF THE INSECT EGG

1. *Introduction.*

I have decided to consider the organization of the egg only in this discussion, since it contains everything neces-

[20] Reagan, 1916, Abstracts 14 annual meeting Amer. Soc. Zool.

sary for the production of a complete organism. In many species of insects and other animals, parthenogenesis is a normal phenomenon and in many species whose eggs must ordinarily be fertilized development may be initiated by artificial means.[21] Among these are the eggs of the silkworm moth. It also seems certain that the cytoplasmic regions of the insect egg have reached a high state of morphological and physiological differentiation before fertilization, judging from the results outlined in Part I. of this paper. The cleavage nuclei may possibly exert an influence upon the cortical layer of cytoplasm before the blastoderm is formed, thereby enabling the paternal contribution to the zygote to act, but besides being very improbable, such a phenomenon would, of course, follow rather than precede the establishment of the advanced state of organization that exists in the undeveloped egg.

2. *Constitution of the Primordial Germ Cells*

In certain beetles, flies and parasitic Hymenoptera, the primordial germ cells are visibly different from the rest of the embryonic cells that arise at about the same time. This difference is primarily due to the inclusion within them of visible substances that are located in the egg material from which they originate. The eggs of these different insects are similar in certain respects and different in others. In every instance, however, this visible substance, which forms the germ-line determinants, is situated near the posterior end of the egg, and it is at this point that the primordial germ cells are formed. The origin of the germ-line determinants is not definitely known in any insect, but their position in the egg and their granular appearance are constant.

It has often been pointed out that the primordial germ cells remain in a comparatively undifferentiated state until the individual in which they lie has almost reached maturity, and that they then undergo changes during

[21] Loeb, 1913, "Artificial Parthenogenesis and Fertilization," Chicago.

which they reach a high state of specialization. The discovery of axial gradients of metabolism in the eggs of certain animals in an anterior-posterior direction[22] suggests that this may also be true of insect eggs. If such gradients exist in insect eggs and if the metabolic activity decreases gradually from the anterior to the posterior end, then the primordial germ cells, which arise at the extreme posterior end, are actually the least active metabolically of all the cells of the embryo. Their early separation from the egg would also tend to keep them in an undifferentiated condition since they are on this account less likely to be influenced by the rest of the embryo.

The primordial germ cells in these insect eggs are thus visibly different because of the presence of germ-line determinants and are probably physiologically different, at least in part, because of their position at the posterior end of the egg.

The contents of these cells are as follows (Fig. 4): (1) part of the cortical layer of cytoplasm, (2) part of the cytoplasm which surrounds the cleavage nuclei and which is collected from among the yolk globules, (3) part of the germ-line determinants, and (4) a nucleus with the full amount of chromatin. The fourth item is mentioned because in *Miastor* all of the nuclei that form somatic cells undergo a diminution process, being similar in this respect to *Ascaris*. This chromatin is in *Miastor* entirely maternal since the eggs of this fly that have been studied, develop parthenogenetically.

Nothing very definite has been discovered regarding the arrangement of these substances in the germ cells. The nucleus lies near the center in all of them; the two kinds of cytoplasm soon become indistinguishable; and the germ-line determinants may, at first, be more or less evenly distributed throughout the cytoplasm, as in chrysomelid beetles and *Miastor*, or may be clumped in various parts of the cell, as in *Chironomus*. In every case, however, the germ-line determinants evidently become

[22] Child, 1916, *Biol. Bull.*, Vol. 30.

more or less evenly scattered since they cannot be distinguished in later stages in the germ-cell cycle.

3. *Differential Divisions during the Formation of Nurse Cells*

There is no evidence of any definite localization of substances or physiological processes in the primordial germ cells when formed, nor do these cells exhibit recognizable polarity or symmetry of any kind. As described in preceding pages, they multiply; migrate into or are enveloped by the tissues of the embryo; separate into two groups from which the ovaries on either side of the body arise; and then pass through another period of multiplication. This brings them to the stage just preceding the growth period. At this time phenomena occur in the ovaries of certain species of insects that have a direct bearing upon our problem; these are concerned principally with the differentiation of oocytes and nurse cells. In *Miastor* the nurse cells are mesodermal in origin, and a group of nurse cells and one oocyte become enclosed within a sheath of epithelial cells (Fig. 8). As the oocyte increases in size it elongates, and then for the first time in its history exhibits recognizable polarity; the anterior end adjoining the group of nurse cells. Polarity may, however, have been present from the time the primordial germ cell was first formed, corresponding to that of the parental egg. The germinal vesicle soon becomes eccentric, but whether or not this indicates that bilateral symmetry has also been

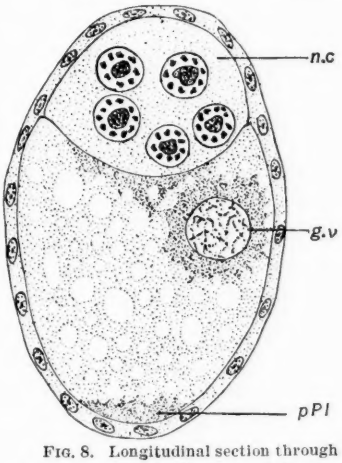

FIG. 8. Longitudinal section through a young oocyte of *Miastor* and its accompanying nurse cells (*nc*). *gv* = germinal vesicle. (Hegner, 1914.)

determined, as it does in certain other insects, is unknown. It is thus certain that polarity exists soon after the beginning of the growth period and that bilaterality is probably also established at an early stage.

The differentiation of oocytes and nurse cells in dytiscid and gyrinid beetles is of peculiar interest, although the early and later history of the germ cells in these insects is not known. In the diving beetle, *Dytiscus marginalis*,[23] a single oogonium gives rise to fifteen nurse cells and one oocyte. The oocyte and its mother cell, grandmother cell, and great-grandmother cell can be dis-

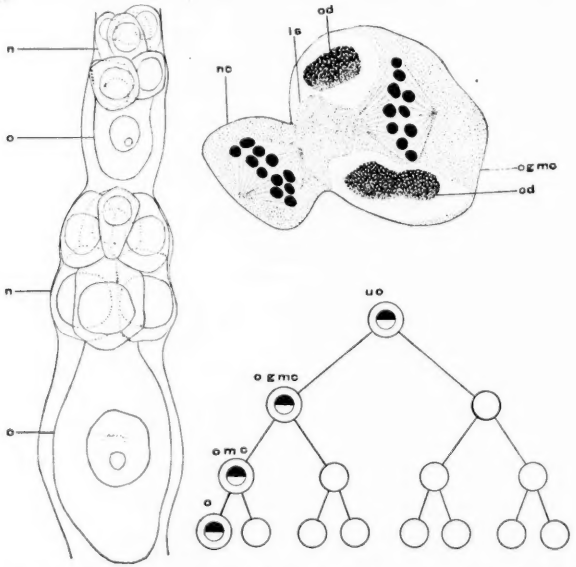

FIG. 9. Diagrams illustrating differential divisions during the formation of nurse cells in the whirligig beetle, *Dineutes nigrior*. (Hegner and Russell, 1916.)

a. Part of the growth zone of an ovarian tubule showing two oocytes (*o*) each accompanied by seven nurse cells (*n*).

b. Division of two-cell stage. An ultimate oogonium has divided forming a nurse cell (*nc*) and an oocyte grandmother cell (*ogmc*) containing the oocyte determinant. *is* = intercellular strand.

c. Diagrammatic representation of oocyte differentiation. The plain circles indicate nurse cells. *uo* = ultimate oogonium containing the oocyte determinant within its nucleus. *ogmc* = oocyte grandmother cell. *o* = oocyte.

[23] Giardina, 1901, *Internat. Monatssch. f. Anat. u. Phys.*, Bd. 18; Debaisieux, 1909, *La Cellule*, T. 25; Gunthert, 1910, *Zool. Jahrb.*, Bd. 30.

tinguished from the nurse cells by the presence of a peculiar ring of nuclear material within the cytoplasm and by their larger size. The gyrinid beetle, *Dineutes nigrior* (Fig. 9), resembles *Dytiscus* in general, but the ultimate oogonium passes through one less division, thus giving rise to one oocyte and only seven nurse cells.[24] The important fact is that during these differential divisions, in both cases, the nurse cells, which may be considered somatic since they are unable to reproduce, are deprived of part of their nuclear material. Apparently they differ from their sister cell, the oocyte, in this one respect, and it is therefore the presence of this nuclear material that makes it possible for the oocyte to develop into a new individual. This is one of the most striking cases of the passage of large masses of nuclear material into the cytoplasm. No such differential divisions have been discovered in chrysomelid beetles nor in the other insects where the nurse cells arise from oogonia, but they may occur in some way that has not been revealed by our methods of research.

The writer has discussed this subject rather fully with relation to the origin of nurse cells and oocytes in the honeybee.[25] In this insect a single oogonium gives rise to a rosette-like group of cells that are connected with one another by strands—probably of a mitochondrial nature—the remains of preceding mitotic divisions. There is no visible difference among the cells in a rosette which are hence apparently potentially alike. Nevertheless one or several from each rosette enlarge to form oocytes which are nourished by the rest acting as nurse cells. What determines the differentiation of certain cells into oocytes is not known but the following hypotheses have been expressed.

Three explanations have occurred to me: (1) There may be differential changes during the mitotic divisions in rosette formation as in *Dytiscus* resulting in one or more cells (oocytes) which differ in con-

[24] Hegner and Russell, 1916, *Proc. Nat. Acad. Sc.*, Vol. 2.

[25] Hegner, 1915, *Journ. Morph.*, Vol. 26.

tent from the others (nurse cells). No visible changes of this sort were observed. (2) The polarity of the rosettes may influence the cells in such a way that those near the center of the ovariole and closest to the zone of differentiation tend to develop into oocytes. (3) Those cells of the rosettes which reach the zone of differentiation first are stimulated to become oocytes and by their growth and differentiation prevent the other cells of the rosettes from similar changes.

4. *Constitution of the Oocyte at the Beginning of the Growth Period*

Very soon after the nurse cells are formed and the oocytes begin to enlarge the main axis of the oocyte in all

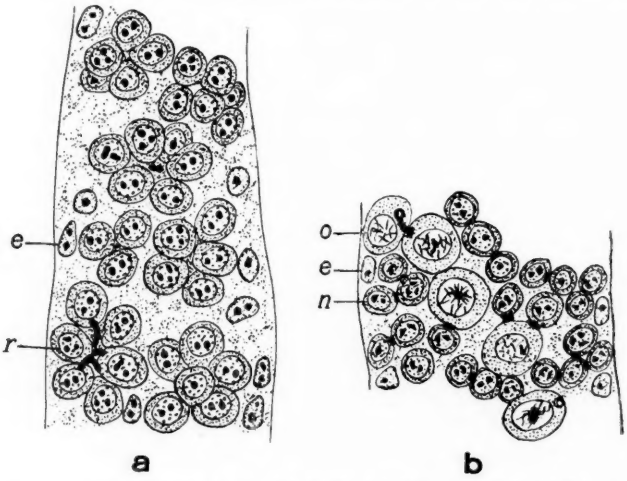

FIG. 10. The formation of oocytes in the honeybee. (Hegner, 1915.)

a. Part of an ovariole showing the rosettes (*r*) each resulting from the division of a single oogonium.

b. Part of an ovariole in the zone of differentiation showing five oocytes (*o*), many nurse cells (*n*) and epithelial cells (*e*).

insects seems to be established. The germinal vesicle at the same time changes its position from the center of the cell to a point near the nurse-cell chamber at the anterior end as described above in *Miastor* (Fig. 8). At what stage bilateral symmetry becomes fixed has not been determined.

The oocytes of insects at the beginning of the growth

period differ from the other cells in the body in the following ways. (1) In all cases where germ-line determinants occur the oocytes alone are provided with them and with the cytoplasm in which they are embedded. (2) In insects like *Miastor* a full amount of chromatin is present only in the oocytes. (3) In *Dytiscus, Dineutes* and probably other insects the oocytes contain nuclear material of which the nurse cells are deprived, but this may be interpreted simply as a means of inhibiting the reproduction of the latter and of changing them into nurse cells. (4) The oocytes seem to have no influence upon the development of the individual in which they lie, as indicated by castration and transplantation experiments,[26] and are in a comparatively undifferentiated condition when the growth period begins.

[26] Meisenheimer, 1912, Fest. 60 Geburtstage von Dr. J. W. Spengel III.; Kopec, 1911, *Arch. Entev.-mech.*, Bd. 33.

(*To be continued*)

INHERITANCE OF FERTILITY IN SOUTHDOWN SHEEP[1]

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INTRODUCTION

YOUATT (14)[2] says:

The disposition to twinning is undoubtedly hereditary:

> "Ewes yearly by lambing rich masters do make:
> The lambs of such twinners for breeders go take."

Flockmasters of the last century have made selections on this assumption, while the increased number at a birth in the progeny of ewes born multiparously as compared to the progeny of those born singly has been demonstrated by several investigators. In general there has been shown to be an increase in number produced at a birth as the average birth values of the animals lambing increase. Thus, Rietz and Roberts (13) present the following in Shropshires, the number at a birth being represented by the figures 1, 2, or 3:

Sire	Dam	Offspring	No. Cases
1.....	1	1.3452±0.0059	3,059
2.....	1	1.3946±0.0073	2,088
1.....	2	1.4171±0.0067	2,436
2.....	2	1.4548±0.0088	1,550
One parent a triplet		1.6076±0.030	158

Experimental investigations of the inheritance of twinning in sheep have been attempted in few cases. Ains-

[1] Paper No. 5 from the Laboratory of Animal Technology, Kansas Experiment Station.

[2] Reference is made by number to literature cited at close of paper.

worth-Davis and Turner (1) reported a preliminary investigation on this subject, but their numbers are too small and results too contradictory, as published, even to be indicative of the method of inheritance. Arkell and Jones (7) at the New Hampshire station also instituted investigations along this line, but have published no results.

Due to the environmental and physiological factors involved in multiple births, as well as to the economic impracticability of maintaining large flocks under rigid experimental conditions, there are at hand no considerable masses of experimental data which yield evidence on this point, nor are the probabilities great that such experiments will ever be conducted on an adequate scale; hence the bulk of evidence on the inheritance of fertility must come from breeders' flocks or from breed registry records.

The Fertility Problem

High fertility obviously depends on three factors—the number at a birth, the frequency of reproduction, and the total number of successful gestations an animal may undergo. Unfortunately flock book records give available data on the first point only, although for specific cases some evidence on the second point (barring abortions and unregistered progeny) exists.

For breeding purposes the number of successful gestations is not a practical selective index, since the breeder can not afford to withhold progeny from breeding until their dams or sires shall have completed their breeding cycles. Frequency of reproduction or regularity of breeding as termed by the breeder is a more practicable trait for purposes of selection, but since barren reproductive periods are so much more frequently due to pathological or physiological causes than to genetic, most sheepmen lay principal emphasis on the number of offspring at the given birth.

There are two ways in which selection on this basis may be applied. The ewe may be selected on the basis of

a particular lambing, or the basis of the best lambing she shows. From a genetic standpoint the second criterion would seem the better, but practical breeders would be very likely to use the first. Unfortunately, records in Southdowns on which a comparison can be based are few, forty-three animals only being available. Table I presents the correlation of each individual record with the average lambing record for each ewe, while Table II presents the correlation of the best record for each ewe with her average. Nine of the ewes had four lambings to their credit, nine had three, while twenty-five had only two. The inadequacy of these data is recognized, since there is a false agreement between a single number and its average with another as compared to its agreement with its average with several numbers. Since, however, the material is suggestive from a comparative standpoint, it is presented, as the same actual error exists in each table:

TABLE I

CORRELATION OF INDIVIDUAL LAMBING WITH AVERAGE LAMBING PER EWE

Average Lambing per Ewe

Individual	1	1.5	1.67	1.75	2	2.5	F
1	38	6	4	4	1	0	53
2	0	6	8	12	31	1	58
3	0	0	0	0	1	1	2
Total	38	12	12	16	33	2	113

The coefficient of correlation for this table is 0.81806 ± 0.02099.

TABLE II

CORRELATION OF BEST LAMBING RECORD WITH AVERAGE OF EACH EWE

Average Lambing of Ewe

High Record	1.00	1.50	1.67	1.75	2.00	2.50	F
1	16	0	0	0	0	0	16
2	0	4	4	4	13	0	25
3	0	0	0	0	1	1	2
Total	16	4	4	4	14	1	43

The coefficient of correlation for Table II was found to be 0.92354 ± 0.01513.

While both records show a higher agreement with the average than probably exists in actual selections, the fact that the best record is more closely in agreement with the average than a random record makes high production a significant selection standard. The correlation between random records and the best records is presented in Table III.

TABLE III

BEST RECORD OF EWE

Individual Record	1	2	3	F
1	38	14	1	53
2	0	55	3	58
3	0	0	2	2
Total	38	69	6	113

The coefficient of correlation here is 0.6518 ± 0.03665. The relationship is not as great as between either of the records and the average record, as shown in Tables I and II. Since the correlation is not as high, and since an error (false agreement with the average) is introduced into each of the first two tables, it is well to determine whether the difference between the first two correlation coefficients is significant. Using the formula for the standard deviation of the difference between the first two constants presented by Pearl (10): Error of $(x - y) = \sqrt{E_x^2 + E_y^2 - 2\, r_{xy}\, \sigma_x\, \sigma_y}$, where E refers to the error, x to the larger constant, y to the smaller, and r_{xy} to the correlation between x and y. The error of the difference between the correlation coefficients of Tables I and II is 0.01598. Since the difference is .10548, it is greater than three times the probable error, hence it is justifiable to conclude that the highest number at a birth is a better indication of the average fertility of an animal than a random birth, although on the basis of the figures presented the latter relationship is high.

Youatt (14) reports in 1837 that one ewe out of five in the average English flock produced twins, which would give 120 per cent. of lambs as the proportion of English flocks at that time.

Mansell (12) reports 168 per cent. of lambs in 11,668 English Shropshires in 1896, while Humphrey and Kleinheinz (6) from figures on the University of Wisconsin flock made the following breed comparisons:

TABLE IV

Breed	Singles		Twins		Triplets	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
Shropshire	42	23.7	120	67.8	15	9.5
Dorset	10	33.3	20	66.7	0	0.0
Southdown	27	38.0	44	62.0	0	0.0
Oxford	3	13.6	16	72.8	3	13.6
Hampshire	9	31.0	20	69.0	0	0.0
Cheviot	9	31.0	20	69.0	0	0.0

Percentage of lambs as given by Mansell is, of course, only a rough indication of twin-bearers, since ewes having triplet and quadruplet births may be included.

Rietz and Roberts (13) show that 43 out of every 100 births in American Shropshires are multiple births, while they have determined from Heape's (5) statistics of 1895–96 that 64 out of every 100 births in English Shropshires are multiple.

Plumb (12) found in 20,037 Shropshire births 59.2 per cent. were singles, 39.2 per cent. twins, and 1.3 per cent. triplets, all recorded in the American Shropshire Flock Book, 1890 to 1899.

Heape (5), from a study of the birth records of 89,000 ewes in English flocks, presents the following data to show the relative fertility of different breeds of sheep:

TABLE V

Breed	Per Cent. Lambs per Ewe	Per Cent. Twin Bearing Ewes
Suffolk	141.77	52.22
Kent	124.05	31.38
Southdown	109.89	18.67
Hampshire	114.69	24.09
Oxford Down	119.16	35.02
Dorset Horn	123.63	37.55
Shropshire	136.79	46.84
Lincoln	111.10	29.09

The figures for the per cent. of lambs per ewe and the per cent. of twin-bearing ewes do not in all cases check each other, as records of certain ewes were available for the one column but not for the other.

The writers tabulated birth frequencies in Shropshires, Cotswolds and Dorsets with the following results:

TABLE VI

Breed	Singles		Twins		Triplets		Quadruplets	
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.
Shropshire.....	10,585	69.41	4,561	29.91	102	.67	2	.01
Dorset.....	2,143	67.75	956	30.22	57	1.88	7	.22
Cotswold.....	5,523	79.24	1,431	20.53	16	.23		

Dorsets seem to have an exceptionally high percentage of triplets and quadruplets.

Factors Affecting Fertility

Heape (5) in a study of 122,673 breeding ewes, 413 English flocks, suggests five physiological factors that may affect the hereditary expression of fertility. The most important factor according to him is the physical condition of the ewe, which must be vigorous and healthy, especially at tupping (mating) time. The second most important factor is the feeding of the ewe, especially flushing previous to breeding, and careful diet during gestation. The third factor in importance is the district; he cited the fact that the Suffolk in its native country produced 60.46 per cent. of twins, while in Essex it produced only 42.87 per cent. The fourth factor in importance he found to be the age of the ewe; and the fifth, the season of year at which mating occurred.

Carlyle and McConnel (3) at Wisconsin discuss time of mating and age of ewe, factors similar to those mentioned by Heape. In a study of twelve years of records of the station flock at the University of Wisconsin they found that ewes bred early in the season dropped a higher percentage of lambs than those dropped late in the season,

while ewes from three to six years of age seemed to be at the optimum breeding period of their life. Humphrey and Kleinheinz (6) found that two-year-old ewes produced 141 per cent. of lambs and six-year-olds, 191 per cent. Possibly the writers do not understand the tables presented by them, but their calculations on the basis of the data there given would show the following averages at each age:

TABLE VII

Age	Average No. per Birth	No. Cases
2	1.54	53
3	1.56	52
4	1.71	52
5	1.73	57
6	1.92	24
7	1.45	11
8	1.00	2
9	2.00	2

Pearl (9) made a biometric study of the fertility of a long-lived ewe whose breeding record was as follows:

TABLE VIII

		Lambs		Lambs
April,	1806	1	1816	2
	1807	1	1817	2
	1808	2	1818	2
April 3,	1809	3	1819	2
Mar. 29,	1810	3	1820	2
	1811	3	1821	1
	1812	3	1822	1
	1813	3	1823	0
	1814	3	1824	0
	1815	2	Total	36

Assuming that the ewe was about one year old when the first lamb recorded was born, Pearl found that the mean point of the ewe's effective breeding life was 8.57 years, that the median point was 8.17 years and that the modal breeding point, or the point of maximum fertility per unit of time, was at 7.34 years.

Taking into account the seventeen years in which some young were born, the following constants regarding the number of lambs per birth were found:

Mean number of lambs per birth, 2.12 lambs.

Standard deviation in number of lambs per birth, 0.76.

Coefficient of variability in number of lambs per birth, 35.78 per cent.

Marshall (8) found after a study of the lambing statistics for various flocks of Scottish sheep for the years 1905, 1906, and 1907, that the percentage of lambs born was, as a general rule, highest among sheep which had been subjected to a process of artificial stimulation by means of special diet at the approach of the breeding season. In some cases the number of lambs per ewes in the "flushed" flocks was nearly 200 per cent. Flocks which were run upon special pasture upon the approach of the tupping season generally produced a slightly larger percentage of lambs than those receiving no sort of special feeding. Evvard (4) found among range ewes fed the same ration that the fourteen heaviest gaining ewes at time of breeding in his flock averaged 1.8 lambs; the fourteen medium gainers, 1.59; and the fourteen lightest gainers, 1.44.

Relation of Mammæ to Fertility

Alexander Graham Bell (2) conducted an experimental investigation on the relation of the number of mammæ to fertility. An unusually high fertility among a flock of native sheep in Beinn Breagh, Nova Scotia, led Bell to examine the ewes in order to discover some distinguishing mark of the twin-bearing ewe. He found a certain number of ewes with one to two supernumerary nipples in an embryonic, functionless condition. Of these abnormally nippled ewes, 43 per cent. had twin lambs, while of the normally nippled ewes but 24 per cent. produced twins. This apparent correlation between multinipples and increased fertility led to an extended series of experiments to ascertain whether by selective breeding, the supernumerary nipples could be made functional, and whether ewes with additional mammæ in a functional condition were more fertile than ewes with the normal number of nipples.

No difficulty was experienced in obtaining ewes that produced milk from six nipples. These multi-nippled sheep, however, did not prove to be more fertile than normally nippled sheep. In his 1912 paper (2) he states that the indications are that the six-nippled stock will ultimately prove to be twin bearers, as a rule, at maturity.

METHOD OF OBTAINING DATA

The source of the data in the present study was the American Southdown Record, the first twelve volumes being used to obtain cases of triplets, and volumes nine to twelve for twins and singles. The pedigree of each animal was reported into the third generation, recording the numbers of offspring at the birth of each animal. Some records on triplets were also taken from the American Shropshire Record, while Volume 25 was used to determine the ratio of singles, twins and triplets, Volumes 9–12 of the Southdown Flock Book for the same purpose, Volumes 12, 13, and 14 of the Continental Dorset Club Record, and Volumes 11 and 12 of the American Cotswold Record.

RELIABILITY OF FLOCK BOOK DATA

Records of the number at a birth in sheep are probably highly accurate for such material, since there is no observable tendency to discriminate in favor of, or against, recording offspring of multiple births, except the indirect one of lesser development in offspring from multiple births. This would not affect the reliability of the figures presented by the flockmaster, except perhaps to reduce slightly the proportion of multiple births registered. It may be safely assumed that the bulk of the records are accurate, barring clerical error.

THE NUMBER AT A BIRTH AS A GENETIC INDEX

Due to the physiological causes limiting the full expression of the genetic fertility of an animal it is obvious that animals recorded as singles may be potentially twin or

triplet bearers, or that ewes recorded as bearing twins may be genetically triplet producers or better. Hence it may be expected that not all single or twin bearers are alike in zygotic make-up with reference to fertility or that their breeding performance will fall into sufficiently well-defined categories to permit a rigorous Mendelian grouping. The relation between a random lambing record and the average record of ewes was shown earlier in this paper to be high, hence a similar relation might be expected to hold for true genetic fertility, were it measurable, and a random record.

The Data Involved

The Relative Influence of Sire and Dam.—Rietz and Roberts (12) found a mathematically significant effect of the sire on the number at birth as adjudged by the correlation between offspring and sires, although they do not find a similar relation between dams and maternal grandsires. While the authors have not secured correlation coefficients on this point, their averages may be so arranged as to throw some light on the same point. Using pedigrees which were started from animals of single birth, the following comparison between sires and dams is possible.

TABLE IX

Relative Influence of Sire and Dam on Birth Number, from Pedigrees of Animals of Single Birth

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
1,872	1	1	1.29	1,872	1	1	1.29
925	1	2	1.28	570	2	1	1.25
14	1	3	1.43	12	3	1	1.50
5,570	2	1	1.25	925	1	2	1.28
306	2	2	1.34	306	2	2	1.34
10	2	3	1.20	6	3	2	1.17
12	3	1	1.15	14	1	3	1.43
6	3	2	1.17	10	2	3	1.20

Comparison of the records of single, twin and triplet sires mated to single, twin and triplet ewes in the preceding table shows no particular influence of the birth rank of the sire, a fact which is confirmed in Table X, where the average performance of each is given.

TABLE X

BREEDING PERFORMANCE OF THE MALES FROM PEDIGREES STARTED WITH SINGLE BIRTHS

Sires	Mean	Standard Deviation	No. Cases
1.....	1.2864±.00593	.4668	2,811
2.....	1.2776±.01031	.4553	886
3.....	1.3888±.07750	.4875	18

The difference between the breeding performance of the singles and twins is 0.0088 ± 0.0119, which is, of course, not sufficient to be significant. It indicates either that the male has no influence on the number at a birth (the most probable supposition) or that singles and twins in the males are genetically similar. The difference between the breeding performance of the triplets and singles is $0.1024 \pm .0777$ and between the triplets and twins is $0.1112 \pm .07818$, neither of which is significant.

For the ewes the result is not particularly different. Table XI presents the result of this comparison.

TABLE XI

BREEDING PERFORMANCE OF THE FEMALES FROM PEDIGREES STARTED WITH SINGLE BIRTHS

Dams	Mean	Standard Deviation	No. Cases
1.....	1.26365±0.00631	.4635	2,454
2.....	1.29345±0.00893	.4658	1,237
3.....	1.33333±0.06490	.4714	24

The difference between the progeny of ewes born singly and those born twins is $.01349 \pm 0.1093$; between singles and triplets is $.05338 \pm 0.06252$; and between twins and triplets is $.03989 \pm 0.0655$.

TABLE XII

RELATIVE INFLUENCE OF SIRE AND DAM ON BIRTH NUMBER, FROM PEDIGREES OF ANIMALS OF TWIN BIRTH

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
2,805	1	1	1.51	2,805	1	1	1.51
1,294	1	2	1.55	687	2	1	1.57
21	1	3	1.86	19	3	1	1.68
687	2	1	1.57	1,294	1	2	1.55
468	2	2	1.56	468	2	2	1.56
10	2	3	1.60	7	3	2	1.43
19	3	1	1.68	21	1	3	1.86
7	3	2	1.43	10	2	3	1.60

Table XII shows the relative breeding performance of the sires and dams in pedigrees started from twin births.

Treating the sires in pedigrees from twin births as in Table X, Table XIII is produced.

TABLE XIII

BREEDING PERFORMANCE OF THE MALES FROM PEDIGREES STARTED WITH TWIN BIRTHS

Sires	Mean	Standard Deviation	No. Cases
1.....	1.5296 ± .00543	.51659	4,120
2.....	1.5682 ± .00724	.49704	1,165
3.....	1.6154 ± .06435	.48650	26

The difference between singles and twins as sires is $.0386 \pm .0091$; between singles and triplets is $.0858 \pm .0645$; and between twins and triplets is $.0472 \pm .0647$.

The difference between singles and twins is in this case significant, being about 4.2 times the probable error. Further consideration will be given this difference when the ewes are discussed.

Treating the ewes in pedigrees from twin births as in Table XI, Table XIV is produced.

TABLE XIV

BREEDING PERFORMANCE OF FEMALES FROM PEDIGREES STARTED WITH TWIN BIRTHS

Dams	Mean	Standard Deviation	No. Cases
1.....	1.2529±.00581	0.51075	3,511
2.....	1.5551±.00827	0.51583	1,769
3.....	1.7742±.05065	0.41811	31

The difference between singles and triplets is $0.2513 \pm .05098$; between twins and triplets, $0.2191 \pm .05132$; and between singles and twins is $0.0322 \pm .01011$. Ewes from triplet births give significantly larger progenies than ewes from single or twin births, while ewes from twin births give significantly larger progenies than ewes from single births, the last difference being 3.323 times the probable error. It is interesting to observe that both

twin rams and twin ewes are significantly better breeders than singles. Just why this result is obtained here in the face of other contradictory data is difficult to understand.

In order to combine the results of the two types of pedigrees it was deemed advisable to utilize the ratio of 1:4.118 twins to singles discovered by examination of volumes 9 to 12, respectively, in order to have the normal relationship between twins and singles. This involved dividing the numbers of individuals in the twin group or multiplying those in the single group. In the first case errors would be increased, due to the elimination of certain groups, while in the second case errors would be increased due to the exaggeration of differences between the random sample in the pedigrees begun from single births and the normal distribution of such a population. It was deemed best to use the second method, since it permitted the retention of the small groups, hence the ratio 1:4.118 was multiplied by the ratio 3,715:5,311, the numbers of individuals in the pedigrees from twin and single births, respectively, which gave the multiplying factor 5.887 for the pedigrees started from single births. Of course, this result is only suggestive; but it was impractical to record the additional 4,300 odd pedigrees necessary to get a true random distribution. Treated this way, multiplying Table V by 5.887 and adding to Table IX, Table XV is produced.

TABLE XV

No. Cases	Sire	Dam	Ave. No. Progeny	No. Cases	Sire	Dam	Ave. No. Progeny
13,826	1	1	1.33	13,826	1	1	1.33
6,739	1	2	1.33	4,043	2	1	1.33
1,103	1	3	1.51	89	3	1	1.54
4,043	2	1	1.30	6,739	1	2	1.33
2,270	2	2	1.38	2,270	2	2	1.38
69	2	3	1.26	42	3	2	1.21
89	3	1	1.54	1,103	1	3	1.51
43	3	2	1.21	69	2	3	1.26

Treating the sires as in Tables VI and X, Table XVI is produced.

TABLE XVI

BREEDING PERFORMANCE OF THE MALES GIVEN IN TABLE XI

Sires	Mean	Standard Deviation	No. Cases
1.....	1.3340±.00229	.48658	20,668
2.....	1.3308±.00403	.47677	6,382
3.....	1.3318±.2908	.49533	132

The difference between singles and twins is .0032 $\pm$ .0463; between singles and triplets, .0978 $\pm$ .02916; and between twins and triplets, .1010 $\pm$ .02936.

TABLE XVII

BREEDING PERFORMANCE OF FEMALES FROM TABLE XV

Dams	Mean	Standard Deviation	No. Cases
1.....	1.3274 ± 0.00243	0.48261	17,958
2.....	1.3444 ± 0.00345	0.48713	9,052
3.....	1.4128 ± 0.02533	0.49234	172

The difference between singles and twins is found to be 0.0170 $\pm$ 0.00422; between singles and triplets, 0.0854 $\pm$ 0.02545; and between twins and triplets, 0.0684 $\pm$ 0.02556. Several of the differences in Tables XV and XVI verge on significance, being at least three times the probable error.

RELATIVE INFLUENCE OF MALE AND FEMALE IN GRANDPARENTS

From the study of the relative influence of the sires and dams on the progeny it would seem fruitless from biometric grounds to look for transmission through one sex more than the other. Yet logically it would seem that the grandsire and grandam on the dam's side would have a more potent effect on the birth number from the dam than would the paternal grandparents. Studies of this sort are available from the pedigrees. Perhaps the first concern is to determine the relation of the birth rank of the grandparents to that of the progeny. Table XVIII presents this information.

TABLE XVIII

RELATION OF BIRTH FREQUENCIES IN GRANDPARENTS TO BIRTH FREQUENCIES OF PROGENY

Grandsire	Grandam	Ave. Birth Ranks	Ave. Progeny	Standard Deviation	No. Cases
1	1	1	1.6500±.00881	.5451	1740
1	2	1.5	1.7041±.01488	.6030	747
2	1	1.5	1.7065±.01923	.5717	402
2	2	2	1.7500±.03328	.5517	308
1	3	2	1.8095±.08642	.5871	21
3	1	2	2.0000±.07855	.0000	6
2	3	2.5	1.6667±.08297	.5634	21
3	2	2.5	1.7500±.14606	.4331	4

The difference between the average progeny from grandparents $1\,♂ \times 1\,♀$ and grandparents $2\,♂ \times 1\,♀$ is $.0579 \pm .02114$. This is not three times the probable error, therefore the difference is not significant. The difference between grandparents $1\,♂ \times 1\,♀$ and grandparents $1\,♂ \times 3\,♀$ is $.1609 \pm .08687$. This also is less than three times the probable error, hence is insignificant. In fact none of the differences are significant.

To determine whether birth rank in males or females among the maternal grandparents has effect on transmission, they were compared in the same manner as the sires and dams were. The results for grandsires are:

TABLE XIX

RELATION OF BIRTH RANK OF GRANDSIRE TO BIRTH RANK OF PROGENY

Birth Rank of Grandsire	Ave. Progeny	Standard Deviation	No. Cases
1	1.6675±.00760	.5643	2,508
2	1.7209±.01442	.5779	731
3	1.9000±.06399	.3000	10

The difference between singles and twins is $.0534 \pm .0163$; between twins and triplets is $.1791 \pm .06559$; and between singles and triplets is $.2125 \pm .06444$. The difference between singles and triplets is 3.62 times the probable error, while the difference between singles and twins is 3.34 times its probable error.

Treating the dams in the same manner as the sires Table XX is produced:

TABLE XX

RELATION OF BIRTH RANK OF GRANDAM TO BIRTH RANK OF PROGENY

Birth Rank of Grandam	Average Progeny	Standard Deviation	No. Cases
1	1.6615±.00737	.5064	2,148
2	1.7177±.01239	.5979	1,059
3	1.7381±.06034	.5798	42

The difference between singles and twins is .0562 ± .01435; between singles and triplets is .0766 ± .06079; and between twins and triplets is .0204 ± .06159. The only significant difference is between singles and twins, which is 3.88 times the probable error.

The probable errors involved seem to indicate little, hence a comparison by correlation of the maternal grandsire and progeny, and maternal grandam and progeny was instituted. Table XXI presents the correlation for the maternal grandsire, Table XXII for the maternal grandam.

TABLE XXI

CORRELATION OF MATERNAL GRANDSIRE AND PROGENY

Birth Rank Grandsire	Birth Rank Progeny				*f*
	1	2	3	4	
1	954	1,435	118	1	2,508
2	251	431	49		731
3		9	1		10
f	1,205	1,875	168	1	3,249

TABLE XXII

CORRELATION OF MATERNAL GRANDAM AND PROGENY

Birth Rank Grandsire	Birth Rank Progeny				*f*
	1	2	3	4	
1	811	1,254	82	1	2,148
2	381	596	82		1,059
3	3	25	14		42
f	1,195	1,875	178	1	3,249

The coefficient of correlation for Table XXI is .0496 ± .0118, while for Table XXII it is .0382 ± .0118. The difference between the correlations of maternal grandsire

and grandam is $.0114 \pm .0167$, a difference insignificant, hence one can not assume sex linkage.

EXAMINATION OF SHROPSHIRE DATA FROM THE MENDELIAN STANDPOINT

A number of Shropshire pedigrees were tabulated which were all started from triplet births. It had seemed from inspection that triplets might be genetically different from twins and singles, hence the pedigrees were tabulated to discover such a difference if possible. If the maternal grandparents affected the number at a birth from their daughter, then it was possible that certain differences might appear in the pedigrees indicating the genetic effects. The results follow.

TABLE XXIII

RELATION OF BIRTH RANK IN OFFSPRING TO BIRTH RANK IN DAM WHEN THE MATERNAL GRANDPARENTS ARE SINGLES

Offspring

Dam	1	2	3	4	Mean	Standard Deviation
1.....	17	16	17	0	2.00	0.824
2.....	12	10	15	1	2.13	0.894
3.....	0	0	1	0	3.00	0.000

TABLE XXIV

WHEN MATERNAL GRANDSIRE IS A SINGLE AND MATERNAL GRANDAM IS A TWIN

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	15	9	15	2.00	0.873
2.....	10	9	25	2.34	0.825
3.....	5	0	0	1.00	0.000

TABLE XXV

MATERNAL GRANDSIRE A TWIN, MATERNAL GRANDAM A SINGLE

Dam	1	2	3	Mean	Standard Deviation
1.....	8	3	13	2.21	0.912
2.....	7	5	7	2.00	0.858
3.....	1	0	0	1.00	0.000

TABLE XXVI

MATERNAL GRANDPARENTS TWINS

Dam	1	2	3	Mean	Standard Deviation
1.....	4	2	4	2.00	0.895
2.....	9	9	9	2.00	0.817
3.....	0	1	2	2.67	0.417

TABLE XXVII

MATERNAL GRANDSIRE A TRIPLET, MATERNAL GRANDAM A SINGLE

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	0	1	0	2.00	0.000
3.....	0	0	0	0.00	0.000

TABLE XXVIII

MATERNAL GRANDSIRE A SINGLE, MATERNAL GRANDAM A TRIPLET

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	0	0	1	3.00	0.000
3.....	0	0	1	3.00	0.000

TABLE XXIX

MATERNAL GRANDSIRE A TRIPLET, MATERNAL GRANDAM A TWIN

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	0	0.00	0.000
2.....	1	0	0	1.00	0.000
3.....	0	0	0	0.00	0.000

TABLE XXX

MATERNAL GRANDSIRE A TWIN, MATERNAL GRANDAM A TRIPLET

Offspring

Dam	1	2	3	Mean	Standard Deviation
1.....	0	0	1	3.00	0.000
2.....	0	1	1	2.50	0.500
3.....	0	0	0	0.00	0.000

Since all the pedigrees were started from triplets the excess of triplets is so great as unduly to weight the ratios. Inspection of the ratios does not reveal any particular difference in the progeny descended from a particular pair of grandparents, whether the dam is a single, twin or triplet. Since also there seems to be no sex linkage involved it seemed desirable to combine similar matings from the standpoint of birth rank. The totals produced are presented in Table XXXI.

TABLE XXXI

SUMMARY OF TABLES XXIII TO XXX WITH RESPECT TO BIRTH RANK OF MATERNAL GRANDPARENTS

No. Offspring

Birth Rank Maternal Grandparents	1	2	3	4	Mean	Standard Deviation
Both grandparents single...	29	26	33	1	2.07	0.85851
One grandparent a twin....	46	26	60	0	2.11	0.88983
Both grandparents twins...	13	12	15	0	2.05	0.83516
One grandparent a triplet..	1	2	4	0	2.43	0.70855

Confirmation of the previous view that twins and singles are genetically alike, while triplets differ from either, seems to be found in Table XXXI. However, the difference between triplets and the mating where one grandparent is a twin is only 0.32 ± 0.20. This is not three times the probable error, but by consulting Pearl and Miner's (11) table it is found that the chances that the difference is significant are about two and a half to one.

CONCLUSIONS

1. In general sheep of a high birth rank tend to produce offspring of a high birth rank.
2. On the basis of the few data presented, the highest record of a ewe appears to be a better selection standard for high fertility than a random record.
3. The frequency of multiple births in sheep varies with the breed.
4. Physiological factors may exert a marked influence

on heredity, the most important factors being the vigor of ewe, the feeding of ewe, the age of ewe, the season and the region.

5. Apparently no relation exists between high fertility and additional mammæ.

6. In pedigrees started from single births, the birth rank of the sire does not affect the birth rank of the progeny; in pedigrees started from twin births, the effect of high birth rank of the sire is only slightly significant (more than three times the probable error).

7. The effect of birth rank of ewe on the birth rank of progeny is the same as that of the sire except in the case of pedigrees started from twin births when it is slightly greater.

8. No evidence for a sex linkage of fecundity factors occurs in the pedigrees tabulated, as shown by a comparison of the relative influence of progeny of the maternal grandam and the maternal grandsire.

9. Evidence from Shropshire triplet pedigrees suggests that triplets are genetically different from twins and singles, which two are probably genetically alike.

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LINKED QUANTITATIVE CHARACTERS IN WHEAT CROSSES

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SINCE wheat has but 8 chromosomes in the sexual cells and since the parents in the macaroni bread wheat crosses here discussed certainly differ in more than 8 visible characters, it was thought likely that a genetic linkage of some of these might be found. The following study is an endeavor to discover whether or not there is such a linkage between the shape of the head, *i. e.*, ratio of width of head (measured parallel to the face of the head which shows the sinuous furrow formed between the two rows of spikelets) to the thickness (measured parallel to the array of seeds in the spikelet) and the texture (translucency or opaqueness) of the grain. Of the two parents here discussed No. 1, the macaroni wheat, had a much flattened head and very hard translucent grains, whereas the other parent, No. 35 (a bread wheat), had a nearly square head with soft opaque grain.

The seed on the F_1 plants of this cross were all wrinkled and intermediate in texture between the two parents, *i. e.*, they were dull, being neither translucent like the macaroni parent nor opaque like those of the bread wheat parent. In order to make a quantitative expression of hardness the perfectly translucent grains of the macaroni parent were called 100 per cent. hard, the seeds of the F_1 50 per cent. hard and those of the bread wheat parent were called 0 per cent. hard. Since in the seeds of the F_2 and F_3 plants every possible degree of intergradation occurred between the characters of the two parents and since any form of classification adopted would be purely arbitrary, it was decided to make it the most simple possible and place all of the variants into three groups by the following means: a grain that was approximately as hard

TABLE I. CORRELATION BETWEEN $\left\{\frac{\text{WIDTH}}{\text{THICKNESS}}\right\}$ OF HEAD AND TEXTURE OF GRAINS. 1914

Ratio	$\left\{\frac{\text{Width}}{\text{Thickness}}\right\}$ of Head															Average $\frac{W.}{T.}$	Total No. Plants	Character of Grain
	.60 .69	.70 .79	.80 .89	.90 .99	1.00 1.09	1.10 1.19	1.20 1.29	1.30 1.39	1.40 1.49	1.50 1.59	1.60 1.69	1.70 1.79	1.80 1.89	1.90 1.99	2.00 2.09			
Pure No. 1							3	7	12	46	*34*	23	21		4	1.63	150	Hard
1 × 35 F_1						1	1	5	4	*9*	12	3	4			1.56	39	Intermediate
Pure No. 35	1	4	*3*	1	2	1										.88	12	Soft

TABLE II. CORRELATION BETWEEN $\left\{\frac{\text{WIDTH}}{\text{THICKNESS}}\right\}$ OF HEAD AND TEXTURE OF GRAINS. 1915

	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	250 259	260 269	270 279	Average W./T.	Total No. Plants	Character of Grain
Pure No. 1				1		5	8	17	22	36	*55*	55	56	32	3	4	2	1						1.57	298	Hard
Pure No. 1				1	1	23	26	26	39	37	*58*	58	47	21	5	6	1	1		1				1.55	352	Intermediate
1 × 35 F_2			2	8	8	29	40	78	85	55	*100*	88	64	67	3	28	25	7	2	2	1		1	1.55	693	Hard
1 × 35 F_2	2	3	7	28	44	161	196	*204*	175	96	120	68	48	47	1	17	3	4	1	1				1.29	1,226	Intermediate
1 × 35 F_2		5	10	34	61	96	*52*	36	15	11	7	3	1	1										1.11	332	Soft
Pure No. 35		3	14	40	*55*	41	13																	.95	166	Soft

TABLE III. CORRELATION BETWEEN $\left\{\frac{\text{WIDTH}}{\text{THICKNESS}}\right\}$ OF HEAD AND TEXTURE OF GRAINS. 1916

	50 59	60 69	70 79	80 89	90 99	100 109	110 119	120 129	130 139	140 149	150 159	160 169	170 179	180 189	190 199	200 209	210 219	220 229	230 239	240 249	250 259	260 269	270 279	Average W./T.	Total No. Plants	Character of Grain
Pure No. 1				3	1	14	17	20	36	*40*	65	34	20	2	1									1.45	253	Hard
						1	3	1	8	10	*20*	9	2	1										1.50	55	Intermediate
1 × 35 F_3			2	4	5	44	88	153	184	225	*475*	322	291	215	40	140	55	23	4	2	3			1.59	2,275	Hard
1 × 35 F_3			3	9	14	120	198	313	*206*	116	171	53	38	20	1	20	4	5						1.33	1,291	Intermediate
1 × 35 F_3			6	7	19	147	208	*338*	177	66	64	16	8	2		5								1.24	963	Soft
Pure No. 35	1	5	12	25	38	*71*	63	25			1													1.04	241	Soft

as the macaroni parent was called 100 per cent. hard; a grain as soft as the bread wheat parent was called 0 per cent. hard; all grains intermediate between these were called 50 per cent. hard. A plant which produced 60 per cent. hard grains, 30 per cent. intermediate grains and 10 per cent. soft grains would therefore be classified as follows:

$.60 \times 1.00 + .30 \times .50 + .10 \times 0 = .75 = 75$ per cent. hard.

Spotted grains (grains containing well-defined areas of opaque starch in an otherwise translucent grain) such as occur frequently in the macaroni wheat and also among the hybrids were treated as intermediate grains. After classifying the seeds of each plant in this manner all those which were over $66\frac{2}{3}$ per cent. hard were termed hard wheats, those from $33\frac{1}{3}$ to $66\frac{2}{3}$ per cent. hard were classed as intermediate and all less than $33\frac{1}{3}$ per cent. hard were classed as soft.

In 1914 the two parents (Nos. 1 and 35) and F_1 of this cross were grown with the following results:

The ratio, W./T. of the F_1 plants of this cross, is thus seen to be much nearer to the macaroni parent both in average and distribution than it is to the bread wheat parent.

Table II shows this same study made for the F_2 plants, grown in 1915.

A uniform correlation between the hardness of the grain and the ratio of width to thickness of head is apparent in Table II.

Table III gives the results of this same study for the crop of 1916. There is again apparent a marked correlation between the ratio of width to thickness of head and the texture of the grain. It will be noticed that both in 1915 and 1916 the soft (0 per cent. hard) hybrids had a higher average ratio W./T. than the sonora. It should, however, be remarked that the hybrid softs were also harder on the average than the pure number 35, but since

TABLE IV

DISTRIBUTION OF PLANTS WITH REGARD TO THEIR PERCENTAGE OF HARD GRAINS AND THE RATIO OF WIDTH TO THICKNESS OF HEAD

	Pure No. 1 in 1915											Pure No. 1 in 1916										
	Percentage of Hard Grains											Percentage of Hard Grains										
	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46
244																						
240	1																					
239																						
235																						
234																						
230																						
229																						
225								1														
224																						
220						1																
219																						
215																						
214																						
210			1		1				1													
209																						
205																						
204																						
200	1	1		1	1			2	1	1	2											
199																						
195																						
194																						
190	1		1		1			2	2	1			1									
189																						
185	5		1	2	2	3	1	3	6	2	1											
184																						
180	1	2	4	3	1	4	3	2	4	3		2							1			
179																						
175	3	2	4	1	7	5	4	6	9	8	1											
174																						
170	3	2	5	3	3	8	6	10	10	4	1	10	3	3	2	1	1		2			
169																						
165	3	2	2	3	1	1	1	4	2	12	4	4		2		1		1				
164																						
160	6	2	7	4	6	9	8	9	11	10		11	1	5	3	5	2	5	1	1	1	
159																						
155	6	4	2	4	3	4	8	10	1	9	3	8	2	2			1		1			
154																						
150	3	3	5	4	3	2	4	6	11	14	4	25	4	7	5	5	6	4	7	4	4	
149																						
145	2	5	2	1	4	5	6	3	4	10	3	6	2	3	1	1			1			
144																						
140	1	1		1	2	4	2	3	2	4	8	11	9	5		1	1	3	4		1	1

	Pure No. 1 in 1915											Pure No. 1 in 1916									
	Percentage of Hard Grains											Percentage of Hard Grains									
	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46	100 96	95 91	90 86	85 81	80 76	75 71	70 66	65 61	60 56	55 51	50 46
139																					
135	3	2		2	1	2	2	4	5	7	7	13	4	1	1	2			2	2	1	
134																					
130	1	1	1	3		2	2	3	3	8	2	5	4	1	2	1	2	2	1			
129																					
125		2	1	3		2	3	1	4	11	8	11	1	3	1		1					
124																					
120	1		1		1		1			1	1	5	1	1					1			
119																					
115		1	2	2	1	1	1	2	3	16	5	12	3		1				1			1
114																					
110												1						1				
109																					
105				1		2		1	2	9	5	4		1								
104																					
100				1	1					2	4	5	3			1		1				
99																					
95																					
94																					
90											1	1										
89																					
85												1										
84																					
80		1								1					2							

they were opaque and graded insensibly into the condition of pure No. 35 it was considered impracticable to make the arbitrary designation of degrees of hardness any more complex than the three groups used. It should be added, on the other hand, that a number of races were secured which were as soft or softer than the type of No. 35 and a number of races which, to all appearances, were fully as hard as pure No. 1. The inheritance of hardness will, however, be reserved for discussion in a later paper.

The question now arises as to whether this correlation is genetic or physiological. Might it not be caused by the simple fact that poorly filled (with starch) hard grains will give rise to a more flattened head, than will plump (starchy, soft) grains by failing to fill up and distend the

glumes? This question can be answered by studying the correlation between the flattening of head and hardness of grain in a pure race. Since all of the No. 35 was soft, this study could not be made for the soft wheat, but as there were many plants of the pure macaroni which produced a greater or less proportion of soft grains, a comparison was possible. That the plants producing a large proportion of hard grains on an average did not have more flattened heads than those which produced a greater proportion of soft grains is shown in Tables II and III by comparing the "hard" with the "intermediate" groups of pure No. 1. This is perhaps better shown in Table IV, where the distribution of the plants is made with regard to their hardness per cent. and the ratio of width to thickness of head.

It should be noted from Tables II and III that there are numbers of individual plants with low ratios of width to thickness of head, but with high percentages of hard grains. It is difficult to see how this could occur if the low ratio was due simply to the lack of plumpness of the hard grain. *Moreover, races of hard macaroni wheats occur which have approximately square heads, and there are varieties of soft wheats (Little Club) with rather strongly flattened heads.* In 1916 there were a few cases where hybrid races having low average ratios (W./T.) also were rather high in average per cent. of hard grains. All of the races in 1916 having ratios averaging as low or lower than 1.35 and average percentages of 60 or more per cent. of hard grains are given in the following table:

It is thus seen that all of the races which on the average violated the correlation in 1916 came from plants which more or less markedly violated this same correlation in 1915. The cases given would indeed be hard to explain on a basis of violation of physiological correlations but if we are dealing with a genetic correlation, they may be easily explained on the "cross over" theory as used by Morgan for reversal of linkages in the characters of *Drosophila.*

TABLE V

INHERITANCE OF NON-CONFORMITY TO USUAL CORRELATION BETWEEN HIGH RATIO W./T. OF HEAD AND AVERAGE PERCENTAGE OF HARD GRAINS

No.	Parents in 1915		Offspring in 1916	
	W./T.	Per Cent H.	W./T.	Per Cent H.
132	.93	98	1.34	88
169	1.10	50	1.24	73
219	1.20	50	1.35	93
232	1.25	100	1.33	86
239	1.33	60	1.30	88
263	1.00	64	1.17	61
279	1.27	50	1.35	79

In the opposite direction, *i. e.*, races which markedly violated the correlation by having very high ratios (1.50 or more) but with low percentages of hard grains did not occur.

Again it may be objected that an explanation of this correlation between shape of head and texture of grain as a genetic linkage, is incorrect because the linkage is not complete, *i. e.*, there is considerable regression. This objection may be fully met by the observation that both of the characters here concerned are quantitative and hence subject to fluctuation around a mean. Moreover, it is almost certain that both characters are genetically compound, *i. e.*, each are the result of more than one factor, recombinations of which may markedly vary the quantitative visible expression of the characters. If, therefore, but a single factor for grain texture be linked with one of the factors concerned in the shaping of the head, there will result a partial correlation of these two characters such as we find.

The data here presented, therefore, seems to indicate that the two characters, hardness of grain and high ratio of width to thickness of head, which entered this cross together in the macaroni parent, tend to come out together in the segregates of the F_2 and F_3 generations, *i. e.*, that there is a genetic linkage between one or more of the factors controlling the grain texture and head shape in the two varieties employed as parents.

ON REVERSIBLE TRANSFORMABILITY OF ALLELOMORPHS

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IN genetical studies of variegation in plants, the fact has been observed occasionally that with a certain frequency a dominant allelomorph occurs in the corresponding recessive homozygote (De Vries,[1] Correns,[2] and Emerson[3]). In this paper the author presents a new instance of a similar phenomenon, which it is hoped may throw additional light on the subject.

In certain pedigree cultures of the rice plant, *Oryza sativa* L., there happened to occur in 1912 families containing besides ordinary fertile plants a number of sterile plants. These sterile plants were normal in their growth, but showed a considerable barrenness at the ripening season. Some of them yielded no seed whatsoever, others bore a small number of normal seeds, and very few were mosaic forms with higher fertility. These families, two in number, each belonging to a different variety, were derived from single plants of the former generation, and were very uniform in other characters. From them the experiment was started.

The rice plant, being a self-pollinated species, is convenient material for breeding experiments. Although the experiments in this investigation were made largely from open-pollinations, the results obtained were always similar to those from experiments in which plants were artificially protected against accidental natural crossing.

The observations of 1912 and 1913 are shown in summarized form in Tables I and II, *a* and *b*, and point to the following conclusions. Sterility behaves as a simple re-

[1] De Vries, H., "Die Mutationstheorie," Bd. I, 1901, pp. 489–511; "Species and Varieties, their Origin by Mutation," 1905, pp. 309–339.

[2] Correns, C., *Berichte der Deutschen Botanischen Gesellschaft*, Bd. 28, 1910, pp. 418–434.

[3] Emerson, R. A., AMERICAN NATURALIST, Vol. 48, 1914, pp. 87–115; *Genetics*, Vol. 2, 1917, pp. 1–35.

cessive to fertility, and the seeds resulting from partial fertility of sterile plants again give segregating families. In Family *A*, which shows an exceedingly slight fertility of sterile plants, the segregation ratio in the offspring derived from fertile individuals is quite close to expectation, but in Family *B* which shows a considerably higher grade of partial fertility of sterile plants, the progeny of fertile individuals exhibit considerable deviations from the expected segregation ratio.

TABLE I

THE SEGREGATING FAMILIES, *A* AND *B*, IN 1912

Fam.	Segregation						Partial Fertility of Sterile Plants		
	Fertile Plants	Sterile Plants	Total No. of Ind.	Steriles %	Ratio per 4		Total No. of Spikelets	Fertile Spikelets	
					D	R		No.	%
							ca.		
A....	36	13	49	26.53	2.94	1.06	9,000	2	0.02
B....	105	25	130	19.23	3.23	0.77	14,941	434	2.90

TABLE II

THE FAMILIES DERIVED FROM FAMILIES *A* AND *B*

(*a*) *The Progeny of the Fertile Plants*

Family in 1912	No. of Families			Ratio per 3		Segregating Families			
	Uniformly Fertile	Segregating	Total	Uniformly Fertile Families	Segregating Families	Fertile Plants	Sterile Plants	Total Number Individuals	Steriles %
A....	10	22	32	0.94	2.06	1,068	346	1,414	24.46
B....	41	64	105	1.17	1.83	4,874	1,301	6,175	21.06

(*b*) *The Progeny of the Seeds on the Sterile Plants*

Family in 1912	Number of Families	Fertile Plants	Sterile Plants	Total Number of Individuals	Steriles %
A.....	2	2	0	2	0.00
B.....	24	401	115	516	22.29

These facts may be interpreted by the following hypothesis. The dominant and the recessive types concerned are assumed to be transformed by certain unknown causes into the other allelomorph. The recessive allelomorph which has made its appearance in Families *A* and *B* is assumed to have originated in the preceding

generation by the transformation of the dominant allelomorph. This recessive state of the hereditary substance, however, has a tendency to revert into the original dominant state. Such reversion is especially likely to occur in vegetative cells, where each recessive allelomorph seems to be able to revert independently. Consequently, in recessive homozygotes the reversion generally will produce heterozygotic cells, either one of the two recessive allelomorphs being changed into the dominant. The heterozygotic cells thus formed will give rise to partial fertility in otherwise sterile plants. Again, the recessive allelomorph in heterozygotic cells may be subject to similar reversion, and such reversion may occur both in the heterozygotic cells of sterile plants and in normal heterozygotes. Here, however, heterozygotic cells will be transformed into dominant homozygotic cells without visible effect on the plant concerned. The consequence of this reversion in the next generation will be that the proportion of the dominant segregates may exceed the theoretically expected figure. Finally, it may be assumed that between Families *A* and *B* there exists a difference in the reverting tendency of the recessive allelomorph, which necessarily will effect the differences in both the intensity of partial fertility of sterile plants and the deviations in the segregation ratio.

In Table III the segregating families derived from the fertile plants of Family *B* are classified according to the magnitudes of the deviations in terms of probable errors. The true percentage for the recessive is assumed, in the one case as 25 per cent. (the Mendelian ratio), and in the other case as 21 per cent. (an arbitrary number). In comparing the two different frequency distributions made in this manner with the theoretical frequency distribution, it is observed that while the frequency distribution of the deviations from 25 per cent. shows a considerable discrepancy from the theoretical, the latter fits the frequency distribution of the deviations from 21 per cent. rather closely, the goodness of fit being $P = 0.915$. Consequently, the *ca.* 4 per cent. deficiency of recessive segre-

gates is a normal expectation and not an experimental error.

TABLE III

THE FREQUENCY DISTRIBUTION OF THE DEVIATIONS IN THE SEGREGATION RATIOS IN THE GROUP OF 64 SEGREGATING FAMILIES DESCENDED FROM FAMILY *B* OF THE YEAR 1912

Dev. /P.E.	−5	−4	−3	−2	−1	0	+1	+2	+3	+4	+5	Total
Experimental frequency (I)....	1	7	14	17	15	6	4					64
Experimental frequency (II)...		1	2	11	19	14	10	5	2			64
Expectation.....	0.2	1.2	4.3	10.3	16.0	16.0	10.3	4.3	1.2	0.2		64.0

Note: In the experimental frequency (I) the true percentage for recessives is taken as 25 per cent., and in (II) as 21 per cent.

Such an aberrant segregation ratio seems to be a constant tendency all through the generations descended from Family *B*. This is shown in Table IV in which the experiments in the years from 1912 to 1915 are summarized.

TABLE IV

THE ABERRANT SEGREGATION-RATIOS OBTAINED IN THE YEARS 1912–1915

Years	No. of Fams.	Parent-plants	No. of Inds.	Fertiles	Steriles	Ster. %	Dev. %	P.E. %	D./P.E.
1912.....	1	Fertile	130	105	25	19.23	5.77	2.55	2.3
1913.....	64	"	6,175	4,874	1,301	21.06	3.94	0.37	10.6
1914.....	10	"	1,560	1,207	353	22.63	2.43	0.74	3.3
1915.....	53	"	4,696	3,732	964	20.52	4.48	0.47	9.5
Total....	128		12,561	9,918	2,643	21.04	3.96	0.26	15.2
1913.....	24	Sterile	516	401	115	22.29	2.71	1.21	2.2
1914.....	34	"	994	779	215	21.63	3.37	0.93	3.6
1915.....	19	"	684	522	162	23.68	1.32	1.12	1.2
Total....	77		2,194	1,702	492	22.43	2.57	0.62	4.1

Again, in regard to the intensity of partial fertility of sterile plants, the descendants of Families *A* and *B* exhibited respectively relations similar to those seen in 1912. (Family *A* was not traced after 1913.) A count of fertile spikelets on sterile plants descending from Family *B* was made in 1914 on 281 plants bearing a total of 101,412 spikelets. In this count the number of fertile spikelets was 3,857, corresponding to 3.78 per cent. of the total number of spikelets. The latter figure may be regarded as the average fertility of sterile plants in the progeny of Family *B*.

The fertile spikelets of sterile plants are generally scattered at random over the panicle, and each fertile spikelet may be regarded as representing a separate case of reversion; but in mosaic forms which show higher fertility and are of rarer occurrence, the reversion may have taken place in earlier stages of plant development, resulting in larger fertile sections. Consequently, when the count of fertile spikelets is made with only the first type of sterile plants, a more correct value for the frequency of reversion may be obtained. The result of such a count on 902 panicles containing 93,635 spikelets is 1,858 fertile spikelets, *i. e.,* 1.98 per cent. of the total number of spikelets.

The mosaic forms appear in several different grades of partial fertility. In a panicle either one or more branches or even one half of the panicle can be highly or entirely fertile, the remaining part being absolutely or nearly absolutely sterile. Similarly, in a single plant some whole panicles can be entirely or highly fertile while others are of the ordinary grade of partial fertility. Furthermore, similar mosaic conditions were also observed in single flowers of sterile spikelets. While all six anthers of a sterile spikelet generally bear none or but few pollen grains, occasionally flowers appear in which certain anthers contain a considerable number of pollen grains of normal appearance and others show the ordinary state of sterility. Hence it may be assumed that the reversion can take place at any stage of plant development.

The partial homozygosity of heterozygotes, corresponding to the partial fertility of sterile plants, may be estimated in the following way. Assuming that the possibility of reversion at any stage of a plant's life, similar to that observed above, may also occur in heterozygotic cells, then we may distinguish for convenience two different types of reversions; there is the reversion which will cause partial homozygosity within a single flower, and the reversion which will produce an entirely homozygotic spikelet or larger homozygotic sectant. Suppose then that the latter reversion will give to the heterozygote

homozygotic (AA) spikelets in any part "x" of the total number of spikelets which is taken as a unit, and again that in the remaining $(1-x)$ part of the total number of spikelets, the other type of reversion will occur, turning some part "y" of the whole generative tissue taken as a unit from the Aa state to the AA state. For simplicity, however, we may substitute "x" for "y" in the above relation, because it seems presumable that a similar probability of reversion may exist constantly all through the plant life. Such a plant will have the following constitution in regard to the generative tissue:

$$x(AA) + (1-x)[x(AA) + (1-x)(Aa)].$$

As the result of self-pollination, the progeny of such a parent plant will show the constitution:

$$x(AA) + (1-x)[\tfrac{1}{4}(1+x)^2(AA) + \tfrac{1}{2}(1-x^2)(Aa) + \tfrac{1}{4}(1-x)^2(aa)].$$

Applying arbitrary values to "x" in this formula, we shall get numerical relations among segregates. In Table V the results of such calculation are compared with results obtained by the experiments in 1913–1915. Thus we may find the average partial homozygosity of heterozygotes around 4 to 6 per cent., the average partial fertility of sterile plants being, as was already shown, *ca.* 4 per cent.

TABLE V

CALCULATIONS ON DATA OF TABLE IV

x %	(AA+Aa)	aa	AA	Aa
4 %	77.88 %	22.12%	38.74%	61.53%
5 %	78.57	21.43	39.69	60.31
6 %	79.24	20.76	40.89	59.11
Observation, No. of Inds.	9,918	2,643	94	135
Percentage	78.96%	21.04%	41.05%	58.95%

It has also been noticed that the sterility concerned is associated with an abnormality represented by the behavior of chlorophyll at the ripening of seeds. While, at the ripening season, the chlorophyll in the fertile sections of the mosaic forms turns to yellow just as in ordinary fertile plants, the chlorophyll in the sterile sections still

remains green. The fertile spikelets occurring in a small number on the otherwise sterile panicle appear on ripening as yellow spots scattered among green spikelets; the plants with both sterile and fertile panicles appear in the fall also as mosaic forms with green and yellow leaves. This feature of the sterile plants is in direct contrast to the behavior of the mosaic plants with the variegated and the entirely green leaves studied by De Vries and Correns.

The observations in the foregoing pages seem to parallel those made by the authors cited at the beginning of this paper. In the present investigation, however, there was observed also the transformation of allelomorphs in the opposite direction, that is, the transformation of the dominant allelomorph into the recessive allelomorph, something scarcely mentioned in the investigations referred to above. The observations in this regard were in brief as follows.

In the first place, the spontaneous occurrence of segregating families was observed again among the descendants of the families which had proved in the experiments already described to be constantly fertile. This suggests, just as did the occurrence in Family *A* and Family *B* in 1912, the probability of the AA cell changing into the Aa cell.

In the second place, a constant tendency of the dominant allelomorph to be transformed into the recessive allelomorph was observed in certain strains. In 1913, special attention was paid to such segregating families in which the excess of recessive segregates over the theoretical expectation was particularly high. Although, as already noted, the variation among the segregating families in 1913 with regard to the deviations from the recessive proportion might possibly have arisen from experimental errors associated with a certain probability of allelomorphic reversion from recessive to dominant, yet it was deemed not impossible that the very considerable excess of recessives exhibited by certain families might be caused by other reasons. This point was seemingly de-

cided by the experiment made with Family *B*80 in 1913 (Table VI), since in this family there was noticed a constant tendency toward the allelomorphic transformation under consideration.

TABLE VI

THE SEGREGATION OF FAMILY *B*/80 AND ITS DESCENDANTS

Year	No. of Families	Parent-plants	No. of Individuals	Fertile Plants	Sterile Plants	Recessives	Deviation of Recessives	P. E.
1913....	1	Fertile	99	69	30	30.30%	+ 5.30%	2.95%
1914....	10	"	1,020	727	293	28.73	+ 3.73	0.91
1915....	5	"	435	309	126	28.89	+ 3.89	1.40
1916....	98	"	11,013	7,832	3,181	28.88	+ 3.88	0.28
Total..	114	Fertile	12,567	8,937	3,630	28.89%	+ 3.89%	0.26%
1914 (*a*).	16[1]	Sterile	199	147	52	23.62%	− 1.38%	2.04%
1914 (*b*).	13[1]	Sterile	100	5	95	95.00%	+70.00%	2.92%
1915....	59[2]	"	548	32	516	94.16	+69.16	1.25
1916....	120[2]	"	1,436	99	1,337	93.11	+68.11	0.77
Total..	192[3]	Sterile	2,084	136	1,948	93.47%	+68.47%	0.64%

[1] Derived from the family in 1913, *i. e.*, Family *B*/80.

[2] Derived from the group (*b*) in 1914.

[3] Excluding the group (*a*) in 1914.

In Table VI there is beside the *ca.* 4 per cent. excess of recessives in the families derived from fertile parents, a remarkable excess of recessives in the families descended from the sterile parents in the group (*b*) in 1914. The sterile plant of this type could not be distinguished from those which, as was shown in Table IV, gave segregating families with an excess of dominants in the intensity of the partial fertility as well as in the behavior of chlorophyll at the ripening of the seeds. Consequently, it may be presumed that although these two types of sterile plants have the same genetical constitution originally, the dominant allelomorphs resulting from the reversion of their recessive allelomorphs are of different stabilities in the dominant state; that is, in the first type of sterile plants such dominant allelomorphs are very easily re-transformed into the recessive state, while in the second type the corresponding dominant allelomorphs tend to remain in the reverted condition.

Corresponding to the excess of recessive segregates, a deficiency of dominant homozygotes among dominant seggregates was also noticed. Among 153 families derived from fertile plants in the experiment above mentioned, 40 families were uniformly fertile, the remaining 113 families showing segregation. The former, therefore, is 26.14 per cent. of the total number of families, and shows 7.19 per cent deficiency from the theoretically expected percentage, 33.33 per cent., the probable error being $\pm$ 2.68 per cent.

In conclusion it may be stated that the allelomorphs concerned in this investigation are probably subject to reversible transformations, and that the probable frequency of the allelomorphic transformation may be practically constant in a certain strain, and possibly may be different in different strains. As to the conditions under which such allelomorphic transformations take place, nothing is yet certain except that these conditions are of a hereditary nature. The manner in which different intensities of allelomorphic transformations are inherited will be the subject of further investigation.

A word may be added here regarding the conception of dominance and recessiveness. Bateson's theory of "presence and absence of factors" is sometimes understood in the sense that the dominant allelomorph is regarded as due to the real presence of an hereditary material unit which is absent in the recessive allelomorph. Such a conception is not in full accordance with the idea of the reversible transformability of allelomorphs as described in this investigation. There is another possibility of the nature of allelomorphs. The dominant and the recessive allelomorphs may be supposed to represent two alternative conditions or phases of a single hereditary substance, somewhat resembling the chemical conception of polymerization. Consequently, the interchangeability between the dominant and recessive allelomorphs is not improbable theoretically.

BUSSEY INSTITUTION,
August 26, 1917

NOTES AND LITERATURE

MUTATIONS IN DROSOPHILA BUSCKII COQ.[1]

Two mutations in eye color have appeared in my cultures of *Drosophila busckii.* These mutations are of especial interest in that, as far as the writer has been able to learn, they are the first that have been recorded in this species. This is the eighth species of *Drosophila* in which mutations have been recorded, the other seven being *ampelophila, repleta, confusa, tripunctata, virilis, obscura,* and *similis.*

The eye mutant which is brighter than normal has been called "red" and the other which is darker than normal has been called "chocolate." The normal eye of this species is darker than that of *Drosophila ampelophila.* The mutant red corresponds very closely to the normal eye color of *ampelophila* except that it is slightly brighter. Ridgeway's "scarlet" (Plate I, color number 5, Ridgeway's Color Standards and Nomenclature, 1912) corresponds most nearly to the eye color of this mutant. In the red eye the central fleck shows as a small round point, while in the normal *busckii* eye, it appears larger and less definite in shape. The red eye darkens with age and closely approaches the normal eye in color, but at its darkest stage it can be distinguished from the normal in that it is less translucent. The chocolate eye is an opaque brown and presents none of the shiny appearance of the normal eye. The central fleck is invisible in newly emerged flies, but becomes more or less distinct as the fly ages. With age the color approaches normal, but always remains slightly darker. Flies over forty-eight hours old so nearly approach normal that they are difficult to distinguish. A newly emerged chocolate corresponds most nearly to Ridgeway's "chestnut brown" (Plate XIV, color number 11, tone m).

The mutation red eye was first observed in November, 1916, and it seems probable that the original mutation occurred somewhat earlier and was overlooked as several red-eyed flies, both males and females, were obtained from this cross. The original stock had been collected about a month earlier in a tomato patch

[1] From the Zoological Laboratory of Indiana University, Contribution No. 157.

near Bloomington, Indiana, and had been bred in the laboratory for two generations.

The mutation chocolate eye was first observed in December, 1916, and here again the original mutation had probably been overlooked as in the cross where the mutation was first observed, several chocolate males and females appeared. The original stock in which this mutation appeared had been bred in the laboratory for three generations and was collected in the same tomato patch where the original red eye stock was collected. The stock in which the red eye appeared was collected on September 19, 1916, and the stock in which the chocolate eye appeared was collected September 14 of the same year and these stocks had been bred as two separate strains when the mutations appeared.

THE GENETIC BEHAVIOR OF RED EYE

Some of the first observed red males were mated to virgin red females and the offspring of this cross were all red flies. This red stock has been kept going for several generations and has given all typical reds. Both red males and red females were crossed with normal wild flies and in F_1 of each cross nothing but normal flies were found. Table I gives the results of the F_2 of these crosses.

TABLE I

F_2 OF RED × WILD CROSS

Red ♀ × *Wild* ♂

Culture Number	Type of Mating	Normal		Red		Total Normal	Total Red
		♂	♀	♂	♀		
332	1 pair	317	322	125	119	639	244
333	" "	305	313	109	107	618	216
334	" "	275	282	100	98	557	198
335	" "	326	299	97	101	625	198
336	Mass	557	552	112	150	1,109	262
Total		1,780	1,768	543	575	3,548	1,118
	Red ♂ × *Wild* ♀						
337	1 pair	340	326	101	105	666	206
338	" "	269	293	79	88	562	167
339	" "	231	253	71	83	484	154
340	" "	244	250	59	86	494	145
341	Mass	469	466	87	100	935	187
342	"	265	228	90	93	493	183
Total		1,818	1,816	487	555	3,634	1,042
Grand total		3,598	3,584	1,030	1,130	7,182	2,160

It can be readily seen that the red eye acts as a non-sex-linked recessive character with the red class falling a little short. Shortage of the mutational class frequently occurs. It seems to make no difference whether the red male or red female is used in the cross. In most cases when mass cultures were made, the expected 3 to 1 ratio was less nearly approximated. The ratio of all the flies examined in the F_2 was 3.32 normals to 1 red.

The Genetic Behavior of Chocolate Eye

A pure stock of chocolate was obtained by mating some of the first observed chocolate males to their virgin chocolate sisters. This stock has bred true for several generations but since the eye changes so rapidly to a color approximating normal, in stocks where the flies are allowed to become more than twenty-four hours old, all gradations between the typical chocolate and normal will be found. The chocolate males and females were bred to wild normals and the F_1 flies were all normal. The results of the F_2 of these crosses are shown in Table II.

TABLE II

F_2 of Chocolate × Wild Cross

Culture Number	Type of Mating	Normal		Chocolate		Total Normal	Total Chocolate
		♂	♀	♂	♀		
Chocolate ♀ × Wild ♂							
402	1 pair	263	212	43	95	475	138
403	" "	154	146	49	60	300	109
405	" "	223	181	57	109	404	166
406	Mass	529	468	67	136	997	203
Total		1,169	1,007	216	400	2,176	616
Chocolate ♂ × Wild ♀							
408	1 pair	257	252	58	66	509	124
409	" "	238	242	45	61	480	106
411	" "	343	312	58	82	655	140
412	" "	83	67	17	17	150	34
413	Mass	812	667	78	161	1,479	239
414	"	426	440	106	133	866	239
Total		2,159	1,980	362	520	4,139	882
Grand total		3,328	2,987	578	920	6,315	1,498

Chocolate eye also acts as a non-sex-linked recessive character with the chocolate class falling considerably below the expected

number. The number of chocolate males, especially, falls low. The totals seem to indicate that this low number of chocolate males in comparison to the number of normal males is partially due to the fact that some of the chocolate males have been called normal, for in practically all of the matings, the number of normal males exceeds the number of normal females. This is unusual, for the writer has examined large numbers of wild *Drosophila busckii* and in a large majority of the cases the number of females has been equal to or greater than the number of males. So it may be that the males approximate the normal color more rapidly than the females and since the flies were examined only once a day, some of the chocolate males were mistaken for normals. Some counts were made, examining the flies twice a day, to test this supposition and they indicated that better ratios could be obtained in this manner. But since the work was completed before this was realized, the difference to be obtained by twice-a-day counts was not thought to be of sufficient importance to require the repetition of the experiments. Also in these matings, the relative number of chocolates was lower where mass cultures were made. The ratio for all of the F_2 flies examined was 4.21 normals to 1 chocolate.

THE GENETIC BEHAVIOR OF RED AND CHOCOLATE WHEN THEY ARE MATED TOGETHER

Red males were crossed to chocolate females and red females to chocolate males and in the F_1 of each cross nothing but normal flies appeared. The results of the F_2 of these crosses are given in Table III.

Here again the number of normal males is considerably above the number of normal females. This could be explained as before, that some of the chocolate males have been mistaken for normals, thus increasing the normal class and decreasing the chocolate class. Since the number in the classes of red and chocolate each fell low in their respective crosses to wild, we can expect the number in these classes to be low in this cross. Taking this fact into consideration, the ratio can be considered a 1:2:1 ratio and gives indication of linkage between the two characters. No red-chocolate double recessives were found, therefore the two mutations may be interpretated as being located in the same chromosome.

TABLE III

F_2 OF RED × CHOCOLATE CROSS

Red ♂ × *Chocolate* ♀

Culture Number	Type of Mating	Normal		Red		Chocolate		Total Normal	Total Red	Total Chocolate
		♂	♀	♂	♀	♂	♀			
450	1 pair	237	206	95	122	86	92	443	217	178
451	" "	149	132	70	56	56	48	281	126	104
452	" "	212	184	78	108	73	83	396	186	156
453	" "	207	186	86	85	70	93	393	171	163
455	" "	246	238	114	129	88	106	484	243	194
456	Mass	373	360	149	153	85	109	733	302	194
Total		1,424	1,306	592	653	458	531	2,730	1,245	989

Red ♀ × *Chocolate* ♂

457	1 pair	232	178	21	22	41	72	410	43	113
458	" "	173	163	62	68	48	59	336	130	107
459	" "	214	178	79	66	66	60	392	145	126
460	" "	186	179	93	99	50	58	365	192	108
461	" "	243	249	94	117	90	94	492	211	184
462	Mass	370	326	121	156	90	129	696	277	219
Total		1,418	1,273	470	528	385	472	2,691	998	857
Grand total		2,842	2,579	1,062	1,181	843	1,003	5,421	2,243	1,846

Since mutations have occurred in eight species of *Drosophila* it seems probable that mutations may be found in all the members of this genus. As to the frequency of mutations, there may be individual variation. The writer's own experience would indicate that mutations occur less frequently in *busckii* than in *ampelophila,* for, during the same period in which the two *busckii* mutations were found, a smaller number of *ampelophila* were examined less critically and six mutations were found.

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SINGING MICE

IN November, 1916, Mr. B. S. York, of Ann Arbor, brought to me a "singing" house mouse that had been captured in his home. This mouse had been heard by members of his family for several weeks, especially late at night and early in the morning. Arrangements were made to carry on breeding experiments with it but it lived only two weeks.

Singing mice have been recorded in a number of publications dating back many years. In 1912 Coburn[1] reported some work he had done with a female singer captured in December, 1911. This individual when mated with an ordinary mouse gave birth to five litters (thirty-three young). None of these were singers and no singers appeared in either the second or third generations. Two other singing mice were described by Coburn in 1913.[2] One was caught in the home of an Italian family in November, 1912, and the other was taken by a farmer in Michigan in March, 1913. Both of these were females.

The Ann Arbor specimen that was brought to me also proved on dissection to be a female. Her song was similar to that reported by Coburn as follows:

> The sound is best described as a rapid whole-toned trill involving the tones *c* and *d*. . . . The quality of the tone resembled somewhat that of a fife or flute, but each tone ended with a slight throaty click.

In every case the song could be heard at least 15 or 20 feet away.

Many causes have been proposed for the presence of this ability to sing such as pregnancy, a diseased condition of the lungs or vocal cords, a parasitized liver, etc. There were no embryos or young in the Ann Arbor specimen and Dr. George R. LaRue was unable to find any parasites that could have induced the singing.

It has been suggested that since all of the singers captured thus far have been females, this characteristic may be sex-linked and due to some structural modification of the vocal apparatus.

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[1] Coburn, C. A., *Journ. Animal Behavior*, Vol. 2, 1912, pp. 364–366.
[2] Coburn C. A., *Journ. Animal Behavior*, Vol. 3, 1913, p. 388.